JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45 WASHINGTON, D. C., OCTOBER 15, 1932 NO. 8

EFFECT OF CARBON DIOXIDE CONTENT OF STORAGE ATMOSPHERE ON CARBOHYDRATE TRANSFORMATION IN CERTAIN FRUITS AND VEGETABLES [1]

By ERSTON V. MILLER, *Assistant Physiologist*, and CHARLES BROOKS, *Principal Pathologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

In an earlier article the writers (*7*) [3] have discussed the inhibiting effect of carbon dioxide on the growth of fruit-rotting fungi and its retarding effect on the ripening processes of fruits and vegetables in storage. The present paper supplements the previous one by presenting the results of chemical analyses of fruits and vegetables treated with carbon dioxide.

WORK OF OTHER INVESTIGATORS

Many investigators have studied the chemical changes that take place in fruits and vegetables after harvest and during storage. Boswell (*5*) has shown that in the ripening processes of pea seeds there occurs a rapid decrease in the percentage of sugar and soluble nitrogenous substances and an increase in starch, total acid-hydrolyzable substances, and insoluble nitrogen compounds (largely proteins). He adds that these changes are so rapid that a slight delay in harvesting results in markedly low quality, and that high sugar and low starch content in young peas is essential for high quality.

Jones and Bisson (*8a*) found that at 0° C. no important changes occur in the carbohydrate content of freshly harvested peas. At temperatures above 0° there is a loss in percentage of the more mobile carbohydrates and an increase in percentage of the more stable carbohydrates. These changes appeared to occur most rapidly at 25°, and more rapidly in shelled than in unshelled peas.

Kertesz (*8b*) reported that changes in the chemical composition of green peas begin immediately after shelling. Important among these changes are loss of sucrose and increase in alcohol-insoluble residue.

A number of investigators have studied the rapid depletion of sugar in green sweet corn after its removal from the stalk. That sweet corn loses a large percentage of its sugar content after removal, especially at summer temperatures, has been demonstrated by Straughn (*11*), Straughn and Church (*12*), Stevens and Higgins (*10*), Appleman and Arthur (*1*), and others. Appleman and Arthur found that the depletion of sugar does not proceed at a uniform rate but is more rapid at first and slows down as it approaches equilibrium, which is reached at the point where about 62 per cent of the total sugar and about 70 per cent of the sucrose have been lost. They state that in general

[1] Received for publication Dec. 18, 1931; issued October, 1932.

[2] The writers are indebted to Howard B. Johnson, junior pathologist, and Oscar J. Dowd, junior physiologist, for assistance in performing the experiments recorded in this paper.

[3] Reference is made by number (italic) to Literature Cited, p. 458.

the rate of loss is doubled for every 10 degrees' increase in temperature between 10° and 30° C. until it reaches 50 per cent of the initial total sugar and 60 per cent of sucrose.

Sweet cherries have been studied by Hartman and Bullis (*8*), who report that in ripening there is an increase in the sugar and solid content of the fruit, a decrease in acidity and astringency, and an increase in specific gravity of both the fruit and fruit juice. Except for loss of weight and volume, there were no striking chemical or physiological changes in the fruit immediately after removal from the tree.

Bigelow and Gore (*4*) conducted studies on the after-harvest changes in "market-ripe" peaches at three different storage temperatures. They divided the actual daily loss in grams of the substance per peach by the solid matter in the flesh at the beginning of storage. Averaging the results of several varieties, they found a loss of "marc," acids, and sucrose at each temperature and in general an increase of reducing sugars.

Appleman and Conrad (*2*), in studies of the pectic constituents of peaches, showed that the conversion of protopectin to pectin could be greatly retarded at low temperatures.

Kidd and West (*9*) reported that an atmosphere of 5 to 15 per cent O_2 combined with 10 to 15 per cent CO_2 about doubles the storage life of apples so long as the temperature is above that associated with low-temperature injury, and that the rate of loss of sugars was about halved under optimum gas-storage conditions as compared with storage in air at the same temperature.

Recently Thornton (*13*) reported on the tolerance of fruits, vegetables, and flowers to CO_2 and discussed the use of the gas for retarding the ripening of bananas and for removing the astringency of pears.

EXPERIMENTAL PROCEDURE

MATERIAL AND TREATMENT

In the present work the fruits and vegetables were treated with various percentages of CO_2 for certain periods of time. Comparable material was placed at each of the temperatures 0°, 5°, 10°, 15°, and 20° C. In some of the experiments a temperature range of 5°, 10°, 15°, 20°, and 25° was substituted. Constant temperatures were maintained by means of the chambers employed by Brooks and Cooley (*6*). There were two lots at each temperature, one receiving the CO_2 treatment and the other not treated and therefore considered as a control. The treated lots were placed in 9-liter glass jars, the ground-glass covers of which had been replaced by 2-hole metal covers sealed with plasticine. The controls were held in glass moist chambers or similar 9-liter glass jars without stoppers.

A constant percentage of carbon dioxide was maintained in the test chambers by means of a continuous renewal of the storage air. The desired concentration of CO_2 was obtained by mixing ordinary laboratory air with CO_2. By means of water displacement the gaseous mixture was driven through copper tubing into the experimental jars in the constant-temperature chambers. The gas was passed through coils of copper tubing within the chambers before entering the jars in order to bring it to the same temperature as the fruit in the jars. By means of a pinch clamp the rate of flow of the gas was so regulated as to permit about 90 gallons of gas to pass through in a period of 24

hours. At the beginning of each experiment the gas was first bubbled through very rapidly until the atmosphere surrounding the fruit had attained the desired concentration of CO_2. The exact concentration of CO_2 employed in the experiment was determined by means of an Orsat gas apparatus, the percentage usually falling a little below the theoretical.

Peas, sweet corn, peaches, and cherries were used for the experiments. Alaska, Gradus, and Nott Excelsior peas were obtained from the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C. Sutton Perfection peas, grown at Mountain Lake Park, Md., were brought to Washington under "top icing." A recording thermometer in the pack indicated that the temperature was about 0° C. while the peas were in transit. Cherries and peaches were procured from the open market, the peaches being in what was apparently the "market ripe" stage of maturity. The sweet corn was obtained either from the Arlington farm or from Falls Church, Va., and was harvested early in the morning and quickly brought to the laboratory.

The amount of material employed for each lot was 250 g of peas in pod, 100 g of cherries, 10 to 15 peaches, and 4 half ears of corn in the "milk" stage. The ears of corn were cut in half transversely, and one half ear with husk was placed in the CO_2 lot and the other half in the control. Thus each lot contained the halves of four different ears. Alternate ears were reversed in order that each lot might receive first the tip half and then the butt half.

When the treatment extended over a period of from three to six days samples were removed every 24 or 48 hours. In these instances over a kilogram of peas and 10 ears of corn were employed for each lot.

SAMPLING

At the end of the CO_2 treatment the lots were removed, tested for flavor, and preserved for subsequent moisture and carbohydrate determinations.

For moisture samples, 3 to 5 g of macerated peas or corn and 5 to 10 g of peaches or cherries were placed in a weighing bottle, weighed, covered with 10 c c of absolute alochol, and dried to constant weight at 70° to 80° C. in a vacuum equal to 25 inches of mercury.

The material for the carbohydrate analyses was preserved in alcohol. The peas were hulled and sampled whole, the cherries were pitted and sliced, the peaches were peeled, pitted, and ground in a food grinder, and two to four rows of sweet-corn kernels were completely removed from each ear by means of a sharp scalpel. The fresh material was dropped into a counterpoised Kohlrausch flask containing 0.25 g of $CaCO_3$, weighed on a torsion balance, and covered with sufficient hot 95 per cent alcohol to make the final mixture 80 per cent. The samples were boiled on a water bath for from three to five minutes. Duplicate samples contained 20 g of peas, 40 g of cherries, 20 or 40 g of peaches, and 16 or 20 g of corn.

CHEMICAL ANALYSES

Sugar was extracted from the sweet-corn samples either by the method of Appleman and Arthur (*1*) or in accordance with the recommendations of the Association of Official Agricultural Chemists (*3*). When the extract was cooled to 20° C. the volume was made up to 200 c c, filtered, and 150 c c was withdrawn for analyses.

With peaches and cherries the supernatant alcohol was decanted off and the sample given three 30-minute refluxings with 80 per cent alcohol. Peas were extracted in the same manner, except that they were ground in a mortar before refluxing. The alcoholic extract of peas, cherries, and peaches was made up to a volume of 500 c c.

The alcoholic aliquots from all samples were evaporated down on a water bath, the alcohol being replaced with distilled water. When cooled again the aqueous solution was transferred to 200 c c flasks, clarified with neutral lead acetate, and the excess lead removed by means of sodium carbonate or potassium oxalate. The volume was made up to 200 c c, and reducing sugars were determined by the Munson and Walker method. Aliquots of 50 c c were reserved for total sugars. In some experiments the total sugar was determined as reducing sugar after inversion with hydrochloric acid and neutralization with sodium carbonate. In other experiments the inversion was made by a commercial preparation of invertase and reported as sucrose. In either case the results were computed as sucrose. Total sugar reported for peaches and sweet corn represents the sum of reducing sugar and sucrose. In peas only traces of reducing sugar were found, and in cherries only traces of sucrose.

The sugar-free residues were retained for determination of acid-hydrolyzable polysaccharides. (Completeness of sugar extraction was determined by the alpha-naphthol test.) The dried sample was placed in a Kjeldahl flask containing 200 c c of distilled water and 20 c c of dilute hydrochloric acid (specific gravity 1.125) and hydrolyzed for three hours in a water bath. The solution was neutralized with sodium carbonate, and reducing substances were determined by the Munson and Walker method. The results were computed as starch and reported as acid-hydrolyzable polysaccharides.

RESULTS

PEAS

The effect of the treatment with CO_2 on the carbohydrate content of Alaska and Sutton Perfection peas is shown in Table 1.

TABLE 1.—*Total sugar and acid-hydrolyzable polysaccharides in peas treated with* CO_2

[Expressed as percentage of fresh weight]

Variety	Treatment		Total sugar		Acid-hydrolyzable polysaccharides	
	CO_2	Temperature (°C.) and time	In treated product	In control	In treated product	In control
	Per cent					
Alaska	44	25°, 1 day	4.6	2.6	5.9	8.7
		20°, 1 day	4.3	3.0	6.5	8.3
		15°, 1 day	4.7	3.1	7.0	8.3
		10°, 2 days	4.2	3.1	6.7	8.1
		5°, 2 days	4.3	4.2	6.9	7.7
Sutton Perfection	42	20°, 1 day	5.8	4.9	2.9	4.2
		15°, 1 day	5.9	5.6	2.6	3.2
		10°, 1 day	6.0	5.7	3.0	3.3
		5°, 2 days	6.1	6.2	2.8	2.6
		0°, 2 days	6.4	6.6	3.7	3.8

With both varieties the CO_2 treatment resulted in the retention of a higher percentage of total sugar and a lower percentage of polysaccharides at all but the lower temperatures. The difference between the treated and control lots is much more pronounced with the Alaska peas than with the Sutton Perfection. A possible explanation of this may be found in the fact that in 1930 this section of the country experienced the worst drought on record, the effects of which were not so evident when the Alaska peas were harvested as later when the Sutton Perfection peas were ripe. In the case of the Alaska peas it will be observed that the treated lots at 10°, 15°, and 20° C. retained 35, 51, and 43 per cent more total sugar, respectively, than did the controls at these temperatures. At 25° there was 76 per cent more sugar in the treated lots than in the controls.

With the Sutton Perfection peas the treated lots at 10° and 15° each contained 5 per cent more sugar than the controls, and at 20° there was 18 per cent more sugar in the treated lots than in the controls.

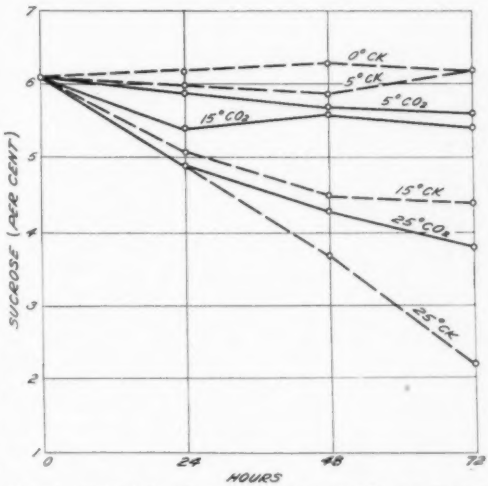

FIGURE 1.—Effect of exposure to 47 per cent CO_2 on sucrose in Nott Excelsior peas at various temperatures. (Solid lines, treated; broken lines, controls)

The results for the Gradus and Nott Excelsior peas appear in Figures 1 and 2, respectively. Both treated and control lots at 5°, 15°, and 25° were compared with a control at 0° C.

The excess of sucrose in the treated lots over the controls is most pronounced at 25° and 15° C. and is more marked as the time element increases. At the end of two days the treated Gradus peas at 15° contained over 10 per cent more sugar than did the controls at the end of the same period. During this time Nott Excelsior peas at 15° contained over 20 per cent more sugar than the controls. The contrast in the case of the Gradus peas was much more pronounced for the 3-day treatment. At the end of three days the flavor of the Nott Excelsior peas at 15° and 25° was objectionable. The low temperature of 5° appeared as effective as the CO_2 treatment in retaining the sucrose in the peas.

SWEET CORN

The results of the CO_2 treatment upon the sugar content of Stowell Evergreen sweet corn will be found in Table 2.

TABLE 2.—*Total sugar in Stowell Evergreen sweet corn treated with CO_2*

[Expressed as percentage of fresh weight]

Temperature (° C.) and time of treatment	35 per cent CO_2		45 per cent CO_2	
	Treated	Control	Treated	Control
20°, 1 day	3.7	3.1	4.1	3.1
15°, 1 day	3.3	3.2	4.3	3.7
10°, 1 day	3.8	3.4	4.2	3.7
5°, 2 days	4.5	4.2	4.3	4.1
0°, 2 days	4.8	5.2	5.3	4.6

It will be noted that with 45 per cent CO_2 the treated lots at all temperatures retained a higher percentage of total sugar than did the controls, the contrast being greater at the higher temperatures. The sweet corn treated at 20° C. contained about 30 per cent more sugar than the untreated lot at the same temperature, whereas at 15° and 10° the treated lots contained over 10 per cent more sugar than the untreated lots. The effect of 35 per cent CO_2 on sweet corn was similar to, though not so great as, that of 45 per cent CO_2.

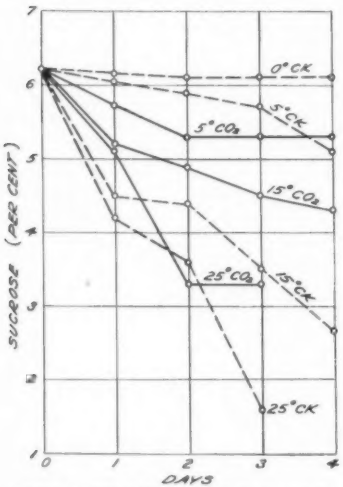

FIGURE 2.—Effect of exposure to 44 per cent CO_2 on sucrose in Gradus peas at various temperatures. (Solid lines, treated; broken lines, controls)

The results for the Early Evergreen and Golden Bantam sweet corn appear in Figures 3 and 4, respectively.

In contrast to the results for peas, the effect of CO_2 on the sugar content of sweet corn was less pronounced at 25° C. than at the lower temperatures. This is especially true for Golden Bantam corn. The general effect of the CO_2 on both varieties was to retard the sugar loss during the storage period. In the results for Early Evergreen sweet corn in Figure 3 it is interesting to note the grouping of the curves for the second day. Apparently the CO_2 treatment was equivalent to a 10-degree drop in temperature for the 25° and 15° lots and a 5-degree drop for the 5° lot.

In the Golden Bantam variety the CO_2 was ineffective in retarding the loss of sugar at 25° C. At 15°, however, there was over 20 per cent more total sugar in the treated than in the untreated lots for the first and second days. On the third day the sugar in the treated lot had dropped to the level of the control. At 5° the treated lots contained over 20 per cent more total sugar than the controls for two, four, and six days, but the corn had begun to lose its palatability on the sixth day.

PEACHES AND CHERRIES

In Table 3 will be found the results of CO_2 treatment of sour cherries, sweet cherries (Bing variety), and Belle peaches. The

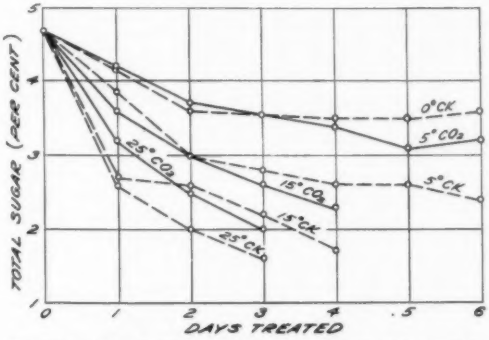

FIGURE 3.—Effect of exposure to 43 per cent CO_2 on total sugar in Early Evergreen sweet corn at various temperatures. (Solid lines, treated; broken lines, controls)

sour cherries received 45 per cent CO_2 for 2 and 4 days; the sweet cherries 44 per cent CO_2 for 2 and 3 days; and the peaches 35, 43, 44, and 47 per cent CO_2 for 1 and 2 days. The variation in

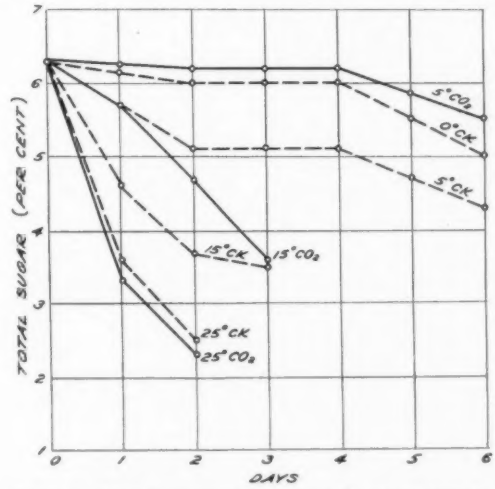

FIGURE 4.—Effect of exposure to 49 per cent CO_2 on total sugar in Golden Bantam sweet corn at various temperatures. (Solid lines, treated; broken lines, controls)

the results with these different percentages of CO_2 on the peaches was very slight, and they have been averaged for the reader's convenience.

TABLE 3.—*Reducing sugar, total sugar, and acid-hydrolyzable polysaccharides in cherries and peaches treated with CO_2*

[Expressed as percentage of fresh weight]

Fruit	Treatment		Reducing sugar		Total sugar		Acid-hydrolyzable polysaccharides	
	CO_2	Temperature (° C.) and time	In treated product	In controls	In treated product	In controls	In treated product	In controls
	Per cent							
Sour cherries	45	20°, 2 days	7.5	6.9			0.62	1.17
		15°, 2 days	7.5	7.3			.53	.69
		10°, 2 days	6.5	7.2			.63	.60
		5°, 4 days	6.5	7.8			.54	.49
		2°, 4 days	7.2	7.2			.55	.59
Sweet cherries (Bing variety)	44	20°, 2 days	12.0	13.2			.68	.88
		15°, 2 days	13.6	12.2			.78	.82
		10°, 2 days	13.0	12.4			.82	.84
		5°, 3 days	11.1	12.2			.73	.75
		0°, 3 days	11.5	11.8			.65	.68
Peaches (Belle)	[a] 5–47	20°, 1 day	2.2	2.2	6.7	7.1	.84	.89
		15°, 1 day	2.2	2.3	6.3	6.3	1.06	.95
		10°, 2 days	2.0	2.4	5.8	7.3	.83	.98
		5°, 2 days	2.2	2.2	7.3	6.8	.85	1.01
		0°, 2 days	2.2	2.0	7.1	7.0	.80	.78

[a] Average of four experiments in which the percentage of gas was 35, 43, 44, and 47, respectively.

Apparently the CO_2 produced no effect upon the carbohydrate content of the peaches and cherries. The percentage of sugar and acid-hydrolyzable polysaccharides remained practically constant for both lots at all temperatures with the sour cherries, sweet cherries, and peaches.

DISCUSSION

The fact that there were no significant effects on carbohydrate transformation in the cherries and peaches as a result of the CO_2 treatment may help to answer a question frequently asked concerning the effect of CO_2 on fresh fruits. In a previous paper (*7*) reference was made to the deleterious effect of too great a concentration of CO_2 on the flavor of certain fruits such as peaches, strawberries, and raspberries. The question naturally arises as to whether the actual food value is also affected. From the evidence presented, the carbohydrate content of peaches and cherries appears to undergo no significant change from the CO_2 treatment given them. It should be recalled that the after-harvest behavior of peaches and cherries is very different from that of seeds like peas and corn. In other words, fruits are not converting their sugar to starch after harvesting, as is the case with peas and corn, and the amount of sugar consumed in respiration does not seem to be influenced by the CO_2 treatment. With the fresh fruits the CO_2 serves its purpose by keeping them firmer for a longer time than would be the case with storage in air.

By far the most pronounced effect of CO_2 on carbohydrate content was noted with peas and corn. It is common knowledge that these vegetables rapidly lose their palatability after being harvested, especially if held at summer temperatures, and this loss of flavor is largely the result of the conversion of sugar to polysaccharides.

The results of this work indicate that the conversion of sugar to starch in peas and corn can be considerably retarded by treatment

with CO_2 of the proper concentration. The greatest effect on peas was noticed at temperatures of 15°, 20°, and 25° C. At 5° and 0° the effect was not marked. Treated Sutton Perfection peas at 20° were 18 per cent higher, and Alaska peas at 25° were 76 per cent higher in total sugar than were the controls. The results for the Nott Excelsior and Gradus peas were similar though not so pronounced.

With all of the sweet corn except Golden Bantam the proper concentration of CO_2 retarded the sugar loss at all temperatures employed. In contrast to the results with peas, the effect of CO_2 on sweet corn was even more pronounced at the lower temperatures. In the experiments on Early Evergreen and Golden Bantam the carbohydrate changes could be easily followed by means of the consecutive sampling of the same lot.

At the time of sampling, the fruits and vegetables were tested for flavor. In all the peaches treated with CO_2 at 15° and 20° C. there was noticed a distinctly overripe flavor; those at 10° were generally questionable or on the border line, whereas at 5° and 0° no effect was noticed. Cherries were generally unaffected by the CO_2 treatment.

Data on the effect of CO_2 on the flavor of peas and corn were obtained by the continual exposure to the gas indicated in Figures 1–4. Forty-seven per cent CO_2 for 2 days did not affect the flavor of Nott Excelsior peas held at 5°, 15°, and 25° C. By the end of 3 days the 15° and 25° lots were objectionable in flavor, but those at 5° were still satisfactory. The Gradus peas at 25° endured 44 per cent CO_2 for 2 days and those at 15° for 3 days without any noticeable effect on flavor. The flavor of the 25° lot was objectionable at the end of 3 days and that of the 15° lot by 4 days of treatment. The 5° lot was satisfactory in regard to flavor by the end of 4 days.

The treatment of the Alaska and Sutton Perfection peas was not continued long enough to obtain data on the effect on flavor.

The Early Evergreen sweet corn reacted similarly to peas under the CO_2 treatment, but the Golden Bantam variety proved a little more sensitive. Both varieties endured the CO_2 treatment (43 to 49 per cent) at 5° C. for 5 days without impairment of flavor. The flavor of the Early Evergreen corn at 5° was questionable at the end of 6 days. The Golden Bantam corn could not be sampled after the fifth day because of insufficient amount of material offered by the normally small ears. The 25° lots of Early Evergreen corn withstood the CO_2 treatment for 2 days and the 15° lot for 3 days without loss of palatability. With the Golden Bantam the limit of treatment was 1 day at 25° and 2 days at 15°.

The treatment of the Stowell Evergreen sweet corn was not continued long enough to obtain data on effect on flavor.

SUMMARY

Peaches, cherries, sweet corn, and garden peas were treated with carbon dioxide gas at different temperatures for periods of one to six days and subsequently analyzed for sugars and acid-hydrolyzable polysaccharides.

The temperatures employed were 0°, 5°, 10°, 15°, 20°, and 25° C. Treatment at the higher temperatures was usually for one to three days, and that at the lower temperatures was usually from two to six days.

No significant difference in percentage of reducing sugar, total sugar, or acid-hydrolyzable polysaccharides was observed when sour cherries, sweet cherries, and Belle peaches were treated with CO_2 in concentrations of from 35 to 47 per cent.

Similar concentrations of CO_2 retarded the rate of sugar loss in peas and sweet corn.

Treatment of peaches with 35 to 47 per cent CO_2 at the higher temperatures (15° and 20° C.) produced a characteristic overripe flavor. In similar experiments the flavor of cherries was not affected by the CO_2 treatment. Exposure to 43 to 47 per cent CO_2 for two days did not affect the flavor of Gradus and Nott Excelsior peas or Early Evergreen sweet corn when held at 25° and 15°. One day at 25° and two days at 15° were the limit of tolerance to CO_2 for Golden Bantam sweet corn. At 5° the flavor of both varieties of peas was normal for three days and that of both varieties of sweet corn for four days. Increasing the time of exposure in any of the above cases was usually detrimental to flavor.

Within the limit of tolerance the treated sweet corn seemed sweeter than the controls when sampled.

LITERATURE CITED

(1) Appleman, C. O., and Arthur, J. M.
1919. carbohydrate metabolism in green sweetcorn during storage at different temperatures. Jour. Agr. Research 17:137–152, illus.

(2) ——— and Conrad, C. M.
1926. pectic constituents of peaches and their relation to softening of the fruit. Md. Agr. Expt. Sta. Bul. 283, 8 p., illus.

(3) Association of Official Agricultural Chemists.
1930. official and tentative methods of analysis . . . compiled by the committee on editing methods of analysis. Ed. 3, 593 p., illus. Washington, D. C.

(4) Bigelow, W. D., and Gore, H. C.
1905. studies on peaches. I. compiled analyses of peaches. II. changes in chemical composition of the peach during growth and ripening. III. effect of storage on the composition of peaches. U. S. Dept. Agr., Bur. Chem. Bul. 97, 32 p.

(5) Boswell, V. R.
1929. factors influencing yield and quality of peas—biophysical and biochemical studies. Md. Agr. Expt. Sta. Bul. 306, p. 341–382.

(6) Brooks, C., and Cooley, J. S.
1917. temperature relations of apple-rot fungi. Jour. Agr. Research 8:139–164, illus.

(7) ——— Miller, E. V., Bratley, C. O., Cooley, J. S., Mook, P. V., and Johnson, H. B.
1932. effect of solid and gaseous carbon dioxide upon transit diseases of certain fruits and vegetables. U. S. Dept. Agr. Tech. Bul. 318, 60 p., illus.

(8) Hartman, H., and Bullis, D. E.
1929. investigations relating to the handling of sweet cherries with special reference to chemical and physiological activities during ripening. Oreg. Agr. Expt. Sta. Bul. 247, 38 p., illus.

(8a) Jones, H. A., and Bisson, C. S.
1932. changes in the composition of the garden pea after harvest. Plant Physiol. 7:273–283. illus.

(8b) Kertesz, Z. I.
1930. the chemical changes in peas after picking. Plant Physiol. 5:399–412. illus.

(9) KIDD, F., and WEST, C.
1930. THE GAS STORAGE OF FRUIT. II. OPTIMUM TEMPERATURES AND ATMOSPHERES. Jour. Pomol. and Hort. Sci. 8:67–77, illus.
(10) STEVENS, N. E., and HIGGINS, C. H.
1919. TEMPERATURE IN RELATION TO QUALITY OF SWEETCORN. Jour. Agr. Research 17:275–284, illus.
(11) STRAUGHN, M. N.
1907. SWEETCORN INVESTIGATIONS. Md. Agr. Expt. Sta. Bul. 120, p. [37]–78, illus.
(12) ——— and CHURCH, C. G.
1909. THE INFLUENCE OF ENVIRONMENT ON THE COMPOSITION OF SWEETCORN, 1905–1908. U. S. Dept. Agr., Bur. Chem. Bul. 127, 69 p., illus.
(13) THORNTON, N. C.
1930. CARBON-DIOXIDE STORAGE OF FRUITS, VEGETABLES, AND FLOWERS. Indus. and Engin. Chem. 22:1186–1189, illus.

RHIZOCTONIA BOTTOM ROT AND HEAD ROT OF CABBAGE [1]

By F. L. WELLMAN [2]

Associate Pathologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The damping-off or wire-stem disease of cabbage (*Brassica oleracea* L.) seedlings, caused by the Rhizoctonia stage of *Corticium vagum* Berk. and Curt., has been known for a long time and has been studied critically by Gratz (*4*).[3] For several years the writer has observed and investigated two other phases of Rhizoctonia injury to cabbage, known as bottom rot and head rot. The disease called head rot was first described by the writer (*13*) in 1927. In 1931 Weber (*14*) reported in abstract a Rhizoctonia bottom rot in Florida.

The present paper reports the results of studies of the cabbage-growing sections of Wisconsin, Indiana, and Illinois and parts of Iowa and Ohio. Each year from 1923 to 1928, inclusive, damping off, or wire stem, and bottom rot have been observed. Head rot, however, has been irregular in occurrence. It was seen first in 1923 and again in 1924. In 1925 it caused losses of 5 to 9 per cent in many fields in northeastern Illinois and southeastern Wisconsin. In 1926 it was less abundant, and in 1927 and 1928 fields were practically free from it.

DESCRIPTION OF RHIZOCTONIA ROTS OF CABBAGE

BOTTOM ROT

Usually there is little trouble from Rhizoctonia until the transplanted seedlings are large enough to begin to shade the ground. The disease first appears on the basal portions of the leaves next to the soil, the ventral side of the midrib often being the first part of the leaf attacked. The resulting lesions are sunken, black, and sharply elliptical, with their long axes parallel to the sides of the midrib. Round black spots appear on the basal parts of the leaf lamina, and these areas gradually enlarge. The diseased spots usually have over them sparse weblike surface mycelia. Eventually there is a general decay of the base of the leaf and the tissues become black and easily torn. The distal part of the leaf finally turns yellow, and the whole leaf droops, may dry up and drop off, and is often covered over by soil during subsequent cultivation. The Alternaria leaf spot (*Alternaria brassicae* (Berk.) Sacc.), which has been described by Weimer (*15*), may be confused with Rhizoctonia. The slowly growing

[1] Received for publication Mar. 30, 1932; issued October, 1932. Cooperative investigations between the Wisconsin Agricultural Experiment Station and the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

[2] The writer acknowledges his indebtedness to Dr. J. C. Walker, of the University of Wisconsin, for criticism and aid in the preparation of the manuscript. He is indebted also to the plant pathology department of the University of Wisconsin and to J. Monteith, jr., formerly of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for cultures of *Rhizoctonia solani* supplied by them for comparison with the writer's cultures.

[3] Reference is made by number (italic) to Literature Cited, p. 469.

lesion produced by the Alternaria becomes light colored and papery and is less extensive than the Rhizoctonia lesion. Rhizoctonia infects the bases of the leaves next to the ground first, whereas Alternaria attacks the upper surface of the tip and other distal points with equal facility. Rhizoctonia bottom rot is not necessarily followed by head rot.

HEAD ROT

Head rot (fig. 1) occurs spasmodically in the field under conditions as yet not well defined. If bottom rot occurs on a headed plant in the field and proper conditions prevail, such as high relative humidity and warm weather, hyphae of the organism grow along the stem without producing decay and infect other leaves farther up. These weaken the midribs and the leaves droop. If feces of cabbage worms lodge on the dorsal side of the petioles and on leaf blades, growth of the organism is stimulated and its head-rotting action is materially hastened. As the bases of the first cover leaves of the head are attacked, the exposed tips on top of the head turn yellow and tend to dry up. A few hyphae spread rapidly between the leaves of the head, and lesions are produced. The latter are mere punctations (fig. 2) at first, but later expand and coalesce and have scant strands of hyphae over them. The lesions are dark and sunken and have a suggestion of concentric zonation about a raised center at the point of infection. Hyphae may later grow out from such spots and produce numerous new lesions near the old. These diseased spots finally become confluent. If the weathered leaves on a diseased head are pulled apart a not unpleasant odor like that of boiled cabbage is noticed. As decay progresses, a weblike dark-colored mycelium develops between the diseased leaves. As the head rot advances only the leaf tissues are affected, while the stem and core of the head are not diseased. The head remains, therefore, conspicuous, upright, dark colored, and studded with small brown sclerotia.

The Corticium stage of *Rhizoctonia solani* Kühn is found early in the cabbage fields of Wisconsin and becomes more prevalent as the season advances. It occurs on the ventral sides of the lower leaves as a superficial gray or chalk-colored membranous growth, which can be easily stripped from its support. It is also found growing over the surface of the soil in protected places and attached to cabbage stems at the ground line.

Comparison with Other Head Rots

The Rhizoctonia head rot differs from other head rots of cabbage in a number of details. The foliage leaves about an attacked head droop in a characteristic manner but may not drop completely from the plant. (Fig. 1, A.) The decaying tissues are more pliable and tough than those produced in watery soft rot or drop (*Sclerotinia sclerotiorum* (Lib.) Mass.) and in bacterial soft rot (*Bacillus carotovorus* L. R. Jones). In Wisconsin two head rots, one caused by Sclerotinia and one caused by Rhizoctonia, produce sclerotia. Rhizoctonia sclerotia are the smaller, the more irregular, and the more numerous. Rhizoctonia decay is neither slimy, as in bacterial soft rot, nor watery and translucent, as in watery soft rot and in head rot caused by species of Pythium (*2*). The infected tissues turn dark and shrink as they decay. In bacterial soft rot the odor of decay is

very noticeable, but in neither of the other head rots mentioned does the odor offend greatly. In Sclerotinia and Pythium rots the aerial mycelium is white and is most abundant in the former. (Fig. 1, B.)

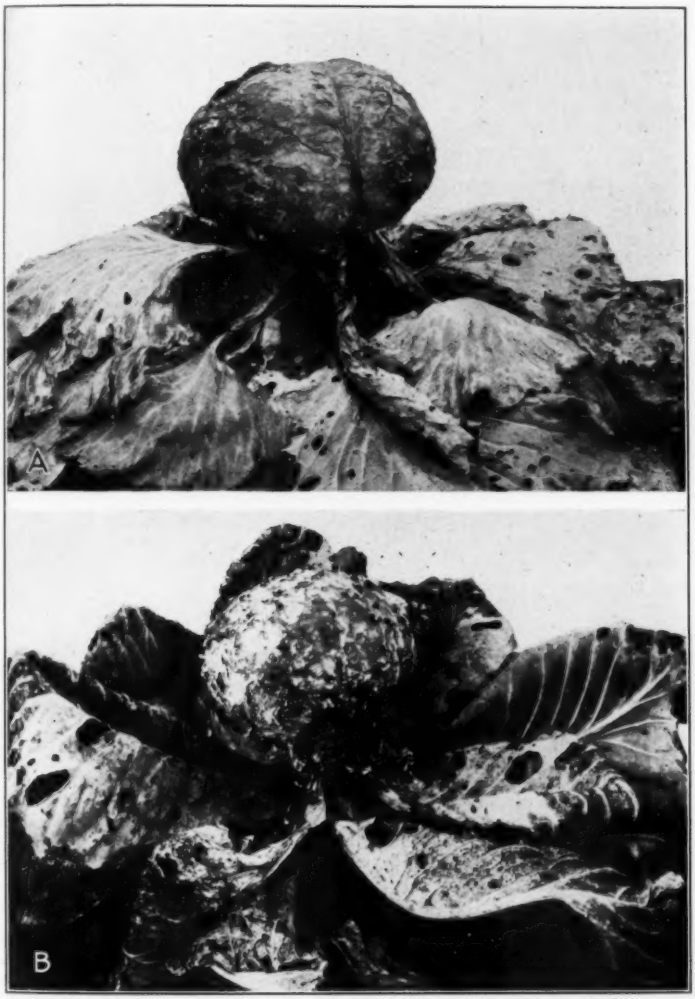

FIGURE 1.—Comparison of Rhizoctonia and Sclerotinia head rots of cabbage in the field. A.—Rhizoctonia head rot: Diseased head erect, somewhat shrunken, roughened, and dark colored; foliage leaves droop but remain attached. B.—Sclerotinia head rot: Some foliage leaves broken off to allow for photographing head; profuse white aerial mycelium; only a few large black sclerotia; leaves not weakened at base as in Rhizoctonia head rot.

Rhizoctonia upon infecting a mature cabbage head attacks only the leaf tissues, whereas bacterial black rot (*Bacterium campestre* (Pam.) E. F. Smith) is characterized by blackened vascular bundles. In cot-

tony rot, watery soft rot, and bacterial soft rot there may be decay of the stem.

THE CAUSAL ORGANISM

ISOLATION

Numerous isolations were made from cabbage seedlings affected with damping off and with wire stem, from leaves affected with bottom rot, and from heads affected with head rot. The Corticium stage of apparently the same organism was commonly found in the autumn on leaves showing signs of bottom rot and around the base of the plants. Four cultures were made from single spores and several

FIGURE 2.—Lesions on surface of inside leaf of cabbage head recently infected with *Rhizoctonia solani*. Older lesions dark and sunken; some show rough concentric zonation around a raised center. Small punctations about large spots are new infections from hyphae advancing beyond older lesions. Such groups of lesions expand rapidly, coalesce, and may involve the whole head. Sclerotia nearly always form at the point of infection.

from bits of the mycelium of the hymenial layer. All these cultures when compared with cultures known to be those of *Rhizoctonia solani* and with descriptions by Kühn (*5*), Duggar (*3*), Matsumoto (*7*), and Burt (*1*), were found to belong to this species.

PATHOGENICITY

Numerous cultures from the sources listed above were used in inoculation experiments upon cabbage plants at various stages of growth, including 15 to 20 flats of young seedlings, a large number of partly grown potted plants, and over 200 cabbage heads, some growing in the field and some cut and placed under bell jars in the laboratory. The results of each experiment were checked with control

plants. The inoculations were generally successful. All strains produced typical damping off of the seedlings, bottom rot of older plants, and typical head rot of heads approaching maturity or already harvested. The results showed conclusively that bottom rot and head rot are caused by the strains of the imperfect stage of the fungus *Corticium vagum* isolated from cabbage. They show furthermore that the causal organism is identical with that causing the Rhizoctonia damping off or wire stem of cabbage seedlings.

PHYSIOLOGIC SPECIALIZATION OF RHIZOCTONIA STRAINS

Physiologic specialization in fungi, in which certain morphologic species are subdivided into forms exhibiting distinct parasitic specialization, is a well-known phenomenon. It has been studied in *Rhizoctonia solani* by several workers, the most important contributions being those of Duggar (*3*), Matsumoto (*7*), and Peltier (*10*). The physiologic reactions of strains of the fungus in pure culture as a measure of biologic specialization has not yet received adequate attention; the pure-culture work of Matsumoto (*7*) and of Monteith and Dahl (*9*) are thus far the most important contributions to this problem.

Physiologic specialization of *Rhizoctonia solani* on crucifers has received only incidental attention. Peltier (*10*) found that a strain isolated from cauliflower, though able to infect seedlings of a fairly wide range of host plants, appeared to attack the Cruciferae most severely. Gratz (*4*), studying strains isolated from a number of host plants, found that the Rhizoctonia causing wire stem of cabbage was probably pathogenic on that plant only, and that the typical potato Rhizoctonia did not cause wire stem of cabbage. Van der Meer (*8*) found that strains of Rhizoctonia obtained from potato and cauliflower "were physiologically distinct from each other." Lauritzen's (*6*) study of Rhizoctonia rot of turnips showed that the potato and turnip strains were pathogenic only on the hosts from which they were isolated. The question of physiologic specialization inevitably presented itself in connection with the present study of Rhizoctonia causing bottom rot and head rot of cabbage.

Sixty-nine isolations of Rhizoctonia from a number of hosts were studied. In this discussion the data from only 15, representing isolations from 6 different hosts, will be considered. The results of comparative inoculations at various stages in the development of cabbage, potato, and lettuce are summarized in Table 1. It is to be noted that the cabbage isolations were practically nonpathogenic to potato and that the potato isolations were nonpathogenic to cabbage. This is in accord with the results of Gratz (*4*) and of Lauritzen (*6*) noted above, indicating that isolations of Rhizoctonia from cruciferous hosts were rather specific to those plants. Isolations from bean, beet, pea, and lettuce likewise failed to cause head rot or bottom rot of cabbage, although that from lettuce did cause damping off of small cabbage seedlings. On the other hand, all the cabbage strains were fairly pathogenic to lettuce. These results, though limited, indicate that only certain strains of *Rhizoctonia solani* are capable of causing head rot and bottom rot of cabbage.

TABLE 1.—*Specialized pathogenicity of certain representative strains of Corticium vagum*

Strain			Results of inoculations[a]									
			On cabbage			On potato				On lettuce		
No.	Host from which obtained	Disease on host	Head rot	Bottom rot	Seedling damping off	Underground shoot decay	Aerial stem lesion	Aerial leaf rot	Sclerotia on tubers	Head rot	Foliage leaf rot	Seedling damping off
1	Bean		—	—	—	—			—	—		+
2	....do	Root rot	—	—	—	—			—	—		—
3	Beet	Dry rot	—	—	—	—						
4	Pea	Root rot	—	—	++	—						
5	Lettuce	Bottom rot	—	—	—	—					+	—
6	....do	Head rot	—	—	—	—				++	++	+
7	Potato	Sclerotia on tuber	—	—	—	—			b	—	—	+
8	....do	Stem lesion	—	—	—	+			b		+	+
9	....do										++	+
10	....do					+++		+++	b			+
11	Cabbage		+	—	—	—		—	b	—	+	+
12	....do	Head rot	+	+	+	—		—	b	+	+	+
13	....do	Bottom rot	+	+	+	—		—	b	+	+	+
14	....do	Wire stem	+	+	+	—		—	b	+	+	+
15	....do	Damping off	+	+	+	—		—	b	—	+	+
		Corticium over stem										

[a] + indicates infection; — indicates no infection.

[b] Sclerotia small and few in number.

ENVIRONMENT IN RELATION TO DISEASE PRODUCTION BY THE CABBAGE RHIZOCTONIA

MOISTURE RELATIONSHIPS

In his studies of damping off Gratz (*4*) found that Rhizoctonia required a high moisture content of the soil. Either drought or excess of water in the soil hindered the development of the disease. The writer's observations corroborate these findings.

To determine the effect of relatively high and low moisture content of the air on the Rhizoctonia producing bottom rot and head rot, a number of experiments were performed. Heads of cabbage obtained on the Madison, Wis., market were taken as host material, and two strains of the fungus were used. After the outer leaves had been carefully removed under aseptic conditions, inoculations at seven points on the top of each head were made from Rhizoctonia cultures. The inoculum consisted of surface sclerotia and bits (about 1 cm square) of the culture medium covered with young growing hyphae. Half of the inoculated heads were placed under bell jars and half were left uncovered in the comparatively dry laboratory air. The temperature of the room was about 21° C. Under the bell jars fungal hyphae could be noted within 24 hours, extending out from sclerotia and bits of agar culture, and in three days the leaf tissue showed symptoms of attack and the organism developed rapidly. Release of water from the host tissue appeared much accelerated with increased decay, water drops gathering in much greater quantities on the inside of bell jars over decaying heads than over uninoculated individuals. On the inoculated heads left uncovered the inoculum dried up and no Rhizoctonia decay resulted.

Plants growing in sterilized soil in the greenhouse were likewise used in determining the relation of moist atmosphere to disease production. These plants, of an early variety of cabbage, had grown rapidly and were forming small heads. Greenhouse temperatures ranged between 18° and 24° C. Inoculations were made with young sclerotia from pure cultures of a cabbage head-rot strain of Rhizoctonia. Inoculum was placed in the forming head and on foliage leaves. Controls were inoculated with bits of sterile agar. The soil was kept wet and some of the plants were covered with a glass-sided box, and others were left uncovered. The Rhizoctonia organism produced typical head rot and bottom rot on all the inoculated plants kept under the moist chamber; a few unprotected plants inoculated in the forming head showed signs of becoming diseased, though the symptoms were not advanced. It appears that a very humid atmosphere is most conducive to the progress of both diseases and is particularly important for the development of head rot.

TEMPERATURE RELATIONSHIPS

Richards (*11*), in 1921, reported studies on the relation of soil temperature to injury of potato stems by *Corticium vagum* (*Rhizoctonia solani*). He found that the organism caused lesions over a wide range of temperatures (9° to 27° C.), that the optimum temperature for pathogenicity was 18°, and that above 24° Rhizoctonia was not a serious factor in disease production. In 1923, he (*12*) again reported studies of Rhizoctonia, this time on pea and bean. As before, he found that temperature had a very definite effect on disease produc-

tion. No infection occurred below 9° or above 29°. The optimum may be placed at 18°. In pure culture the range differed, the minimum temperature for growth being 4.6°, the optimum from 25° to 27°, and the maximum 32.6°. Gratz (*4*), in 1925, found that Rhizoctonia damping off of cabbage had quite definite temperature relationships. The minimum for disease production was below 10°, the optimum from 15° to 30°, and the maximum from 31° to 32°. In pure culture the organism grew at a minimum of approximately 9°, an optimum between 22° and 26°, and a maximum of slightly above 31°. In 1928, Monteith and Dahl (*9*) reported temperature relationships of pure cultures of nine strains of *Rhizoctonia solani* from grass, pea, and potato. In general these strains grew at a minimum temperature of less than 10°, an optimum of 25° to 30°, and a maximum of 35°.

Temperature relationships determined by the writer for the organism causing bottom rot and head rot of cabbage are practically the same as those found by Gratz (*4*). In pure culture on potato-dextrose agar the cabbage Rhizoctonia produced growth at 8° C. At 9° the fungus grew very slowly. Between 12° and 22° rapidity of growth increased enormously. The most rapid growth was above 23° and below 27°. At 29° the rate of growth was materially lessened, at 30° it dropped still further, and at 33° no growth occurred. General field observations indicated that Rhizoctonia was most active during warm weather. Headed plants were potted, inoculated with sclerotia from pure cultures of Rhizoctonia causing head rot, and placed in greenhouses at temperatures ranging from 10° to 33°. Inoculated heads decayed slowly at a temperature of 12°. At 15° to 20° the disease developed at a slightly more rapid rate. At 21° to 23° disease production was further enhanced. The optimum temperature for head rot was determined to be from 23° to 27°. Above 30° decay was considerably lessened, and at 32° there was none.

In a further study of temperature relations, bleached leaves aseptically removed from the inside of heads of market cabbage were put in sterilized moist chambers and inoculated with pure cultures of two strains of Rhizoctonia obtained from cabbage plants affected with head rot. The moist chambers were then held at constant temperatures. Temperature relationships for decay were the same as previously determined, except that the minimum temperature was placed at about 9° C. and the optimum temperature narrowed down to between 25° and 27°.

SUMMARY

The present paper describes two diseases of cabbage, Rhizoctonia bottom rot and head rot, and presents the results of recent studies on both of these diseases, which are caused by *Rhizoctonia solani* Kühn, the imperfect form of *Corticium vagum* Berk. and Curt. Bottom rot attacks cabbage every year, but head rot is spasmodic in its occurrence. It was proved by isolation and inoculation experiments that these two diseases of older plants in the field are caused by the same strain of Rhizoctonia that produces damping off, or wire stem, of cabbage seedlings.

A number of strains of *Rhizoctonia solani* were obtained and studied for evidence of physiologic specialization. It appears that strains of the organism are most pathogenic upon the hosts from which they were originally isolated.

Environmental factors were studied in relation to disease production. It was found that a relatively large amount of moisture is necessary for optimum disease production by the fungus. Rhizoctonia bottom rot and head rot were produced at temperatures ranging from 9° C. to somewhat less than 32°. The optimum temperature for disease development is between 25° and 27°.

LITERATURE CITED

(1) BURT, E. A.
1918. CORTICIUMS CAUSING PELLICULARIA DISEASE OF THE COFFEE PLANT, HYPOCHNOSE OF POMACEOUS FRUITS, AND RHIZOCTONIA DISEASE. Ann. Missouri Bot. Gard. 5:119–132, illus.

(2) DRECHSLER, C.
1925. PYTHIUM INFECTION OF CABBAGE HEADS. Phytopathology 15:[482]–485, illus.

(3) DUGGAR, B. M.
1915. RHIZOCTONIA CROCORUM (PERS.) DC. AND R. SOLANI KÜHN (CORTICIUM VAGUM B. AND C.) WITH NOTES ON OTHER SPECIES. Ann. Missouri Bot. Gard. 2:403–458, illus.

(4) GRATZ, L. O.
1925. WIRE STEM OF CABBAGE. N. Y. Cornell Agr. Expt. Sta. Mem. 85, 60 p., illus.

(5) KÜHN, J.
1858. DIE KRANKHEITEN DER KULTURGEWÄCHSE, IHRE URSACHEN UND IHRE VERHÜTUNG. 312 p. Berlin.

(6) LAURITZEN, J. I.
1929. RHIZOCTONIA ROT OF TURNIPS IN STORAGE. Jour. Agr. Research 38:93–108, illus.

(7) MATSUMOTO, T.
1921. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. XII. PHYSIOLOGICAL SPECIALIZATION IN RHIZOCTONIA SOLANI KÜHN. Ann. Missouri Bot. Gard. 8:1–62, illus.

(8) MEER, J. H. H., VAN DER.
1926. RHIZOCTONIA—EN OLPIDIUM—AANTASTING VAN BLOEMKOOLPLANTEN. Tijdschr. Plantenziekten 32:[209]–242, illus.

(9) MONTEITH, J., JR., and DAHL, A. S.
1928. A COMPARISON OF SOME STRAINS OF RHIZOCTONIA SOLANI IN CULTURE. Jour. Agr. Research 36:897–903, illus.

(10) PELTIER, G. L.
1916. PARASITIC RHIZOCTONIAS IN AMERICA. Ill. Agr. Expt. Sta. Bul. 189, p. 283–390, illus.

(11) RICHARDS, B. L.
1921. PATHOGENICITY OF CORTICIUM VAGUM ON THE POTATO AS AFFECTED BY SOIL TEMPERATURE. Jour. Agr. Research 21:459–482, illus.

(12) ———
1923. SOIL TEMPERATURE AS A FACTOR AFFECTING THE PATHOGENICITY OF CORTICIUM VAGUM ON THE PEA AND BEAN. Jour. Agr. Research 25:431–450, illus.

(13) WALKER, J. C.
1927. DISEASES OF CABBAGE AND RELATED PLANTS. U. S. Dept. Agr. Farmers' Bul. 1439, 30 p., illus. (Section on Rhizoctonia Head Rot prepared by F. L. Wellman, p. 29.)

(14) WEBER, G. F.
1931. BOTTOM ROT OF CABBAGE CAUSED BY CORTICIUM VAGUM. (Abstract) Phytopathology 21:117.

(15) WEIMER, J. L.
1924. ALTERNARIA LEAFSPOT AND BROWNROT OF CAULIFLOWER. Jour. Agr. Research 29:421–441, illus.

THE DIGESTIVE ENZYMES OF THE COLORADO POTATO BEETLE AND THE INFLUENCE OF ARSENICALS ON THEIR ACTIVITY[1]

By DAVID E. FINK [2]

Entomologist, Division of Insect Toxicology and Physiology, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

In a previous investigation the writer (*6*)[3] found that, in general, arsenic caused an inhibition of the respiratory metabolism of insects. This led to the assumption that arsenic may exert its toxic action through its chemical affinity for some substance in the protoplasm that normally is of vital importance to respiration. Glutathione, known to be of importance in cell oxidation and reduction and detected by the writer (*7*) in insect tissue, was found to be reduced in quantity when arsenicals were fed or injected into insects (*8*). Furthermore, Voegtlin, Dyer, and Leonard (*18, p. 304*) have stated that

> arsenic in the form of the trivalent directly toxic oxide modification exerts its action on mammalian protoplasm through its chemical affinity to the SH group of reduced glutathione, thus interfering with the normal equilibrium between oxidized and reduced glutathione, as a result of which the tissue dies of asphyxia.

The foregoing studies indicate that the reaction between arsenic and reduced glutathione may be an important part of the mechanism of the toxic action of arsenic. Parfentjev and Devrient (*10*), however, believe that the effect of arsenic on the respiratory metabolism of insects can not explain the mechanism of its toxic action. They suggest that the loci of its action should be sought elsewhere. Since our knowledge of the mechanism of the toxic action of arsenic is contradictory and incomplete, it seems desirable to exhaust every possible line of investigation before assigning to any one reaction a predominant rôle in the total effect of arsenic.

If arsenic interferes with the activity of respiratory catalysts, perhaps it also affects certain digestive enzymes. A study of the literature failed to reveal a single experiment on the effect of arsenic on the digestive enzymes of insects. Accordingly, the writer has made a study of the effect of arsenicals on the digestive enzymes of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). In this investigation it was necessary first to determine the presence and activity of the digestive enzymes in the normal insect and afterwards to investigate the influence of arsenicals upon their activity.

Received for publication Feb. 15, 1932; issued October, 1932.

[2] The writer expresses his gratitude to J. E. Graf, formerly in charge of the Division of Truck Crop Insects, for the opportunity and encouragement to continue these physiological studies. During the summers of 1928 and 1929 the writer was assisted in the experimental work by H. Beerman, C. E. Herber, and H. Rosen.

[3] Reference is made by number (italic) to Literature Cited, p. 481.

THE DIGESTIVE ENZYMES OF THE NORMAL COLORADO POTATO BEETLE

METHODS OF INVESTIGATION

Active, feeding potato beetles were deprived of food for several days and were given large quantities of water during the same period to clear the digestive tract of food material. Repeated dissections of beetles have shown that a period of two to three days is usually sufficient for the removal of the greater part of the food. The number of beetles dissected at any one time depended upon the extensiveness of the experiment, and ranged from about 100 to 300.

The entire digestive tract was removed and divided into three parts, the fore-gut, mid-gut, and hind-gut. The fore-gut is about 4 mm in length, the mid-gut about 16 mm, and the hind-gut about 8 mm. Dissections were made in a 0.2 per cent sodium chloride solution. Any food and excrement remaining were removed, and the dissected parts rinsed in several changes of the salt solution and finally in distilled water. The three parts of the digestive tract thus prepared were divided into two equal samples. The three parts of the first sample were ground separately with clean washed sand in a mortar. Each part was placed in an Erlenmeyer flask, and the contents of each of the three flasks were diluted with a 50 per cent glycerol solution in the proportion of 1 c c of the solution to 1 mid-gut, 4 fore-guts, and 2 hind-guts. The other sample was similarly treated, except that distilled water was used as the diluent. The tissue suspensions prepared in this manner were fairly homogeneous. A layer of toluene (5 c c) was added to the contents of each of the six flasks as a preservative. The glycerol-tissue suspension was subsequently used in the experimental work on proteolytic enzymes, and the distilled water-tissue suspension was used in the work on enzymes that split carbohydrates. This division was necessary because glycerol solutions in certain concentrations are known to inhibit the activity of certain enzymes.

A suspension was also prepared of the contents of the digestive tract that usually ooze from the mouths of active potato beetles when handled. This regurgitated liquid was collected by means of a fine-tipped capillary pipette held close to the mouths of active beetles. It was then diluted with distilled water to a yellow suspension which contained very active enzymes.

To determine the effect of age of the tissue suspensions on the activity of the enzymes, some experiments were made with a tissue suspension from 1 day to 3 weeks old.

To determine the presence or absence of enzymes, preliminary experiments by the general micro and macro methods described by Swingle (*17*) were tried with these tissue suspensions. Afterwards other methods and modifications were adopted for estimating the extent of digestion caused by a given enzyme. These methods will be described in detail under each enzyme discussed.

A striking characteristic of enzyme activity is the fact that it is limited to a definite range of hydrogen-ion concentration. One of the aims of the writer was to determine the hydrogen-ion concentration of optimum activity and the effective pH range for each enzyme studied in this investigation. In preliminary experiments the writer was guided in the choice of pH range by the large amount of published

information on the effective pH ranges for different enzymes. Clark's (*4, p. 104*) phthalate and phosphate mixtures were employed as buffers. In some experiments the solutions were not buffered but were adjusted to a desired hydrogen-ion concentration by the addition of hydrochloric acid or sodium carbonate. The hydrogen-ion concentrations of the solutions before and during their use in the experiments were frequently checked against those of standard buffer solutions, generally by the usual spot plate and potentiometer methods (*9*).

EXPERIMENTAL WORK

Hydrogen-Ion Concentration of Tissue Suspension

The tissue suspensions in distilled water described in the preceding section were used first for the determination of their hydrogen-ion concentration. Samples were tested by the potentiometer method. The pH of the distilled water was adjusted to 7.0 by means of dilute NaOH. The average pH of the tissue suspension of the fore-gut was 6.42; of the mid-gut, 5.00; and of the hind-gut, 5.40. The average pH of the suspension of regurgitated liquid was 5.00.

Table 1.—*Digestion of starch by amylase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Period of digestion	Extent of digestion [a] at pH of—				
			6.0	6.6	7.0	7.6	8.0
		Minutes					
2	Normal	5	0	-----	20	20	20
		25	20	-----	40	60	35
		50	30	-----	50	70	40
		70	40	-----	60	80	45
		115	60	-----	80	100	60
	Control	-----	0	-----	0	0	0
3	Normal [b]	5	40	20	20	20	-----
		15	60	40	40	40	-----
		25	100	65	50	45	-----
		60	-----	100	80	55	-----
		115	-----	-----	100	80	-----
	Control	-----	0	0	0	0	-----
5	Fed Paris green	5	0	0	0	10	20
		25	0	20	20	20	30
		50	20	30	35	40	40
		70	40	40	60	60	55
		115	60	65	70	75	60
	Control	-----	0	0	0	0	0
6	Fed lead arsenate	5	10	20	20	20	20
		25	20	30	40	40	30
		50	30	40	50	60	35
		70	40	50	70	75	50
		115	60	70	80	85	70
	Control	-----	0	0	0	0	0

[a] Complete digestion equals 100.
[b] Regurgitated liquid was used instead of tissue suspension.

Amylase

Two cubic centimeters of tissue suspension, 2 c c of buffer solution of a desired hydrogen-ion concentration, and 0.5 c c of a 0.5 per cent soluble-starch solution were accurately measured into each of a set of test tubes. A similar set containing boiled tissue suspension was used as a control. These solutions were covered with a layer of toluene and placed in a water bath at 37° C. A drop of an iodine solution (1 per cent iodine-potassium iodide solution) was added

to each test tube. At certain intervals, shown in Table 1, the progress of the hydrolysis of starch by this enzyme was followed by comparing the colors of the solutions with those of a set of five standards ranging from a deep blue to light yellow. The standards were prepared by adding bromothymol blue to buffered solutions of suitable hydrogen-ion concentration. As a result of preliminary experiments, numbers were assigned to the standards as follows: Deep blue, 0; light blue, 20; purple, 40; yellow, 60; light yellow, 80; colorless, 100. The interpolated numbers given in Table 1 indicate estimates of extent of digestion in a digest tube when at the given interval of time the color of the solution in the tube did not match that of a standard. The hydrogen-ion concentration of the solution reaching the achromic point in the shortest interval of time approximated the hydrogen-ion concentration of optimum activity of the enzyme. Before the progress of digestion in a tube was recorded, several more drops of the iodine solution were added to make certain that the color change was due to digestion of starch and not to absorption of iodine.

Enzymes Hydrolyzing Disaccharides

During the hydrolysis of disaccharides by different enzymes, glucose is produced. For example, lactase hydrolyzes lactose into galactose and glucose, maltase hydrolyzes maltose into two molecules of glucose, and invertase hydrolyzes sucrose into fructose and glucose. It was found convenient to take advantage of this fact for the purpose of detecting and estimating the relative activity of the different enzymes hydrolyzing disaccharides. Benedict's quantitative solution (a modification of Fehling's solution) was used. One cubic centimeter of this reagent, when reduced, is equivalent to 2 mg of glucose. After determining the number of cubic centimeters of an incubated mixture required to reduce 2 c c of Benedict's reagent, one is able to express the extent of digestion in milligrams of glucose per cubic centimeter of incubated mixture.

The writer found the following method convenient: Into a series of test tubes 2 c c of Benedict's solution was accurately measured by means of a burette. In addition, each tube received 0.5 g of anhydrous sodium carbonate and a little powdered pumice, and the contents were heated to boiling over a free flame until the carbonate was completely dissolved. By means of a 1, 2, or 5 c c Mohr pipette filled to the zero mark, the incubated mixture to be tested was run into the boiling reagent (the test tube being held just above the tip of a free flame), rather rapidly at first, then more slowly as the color began to fade, and finally drop by drop until a white precipitate formed and the solution became colorless. A pipette graduated to 0.01 c c was used to obtain the end point. The error in determining the end point was not greater than 0.03 c c of tissue suspension. The reduction of 2 c c of Benedict's reagent was accomplished under these conditions by exactly 4 mg of glucose.

The figures in milligrams of glucose are an indication of the relative hydrolyzing activity of the enzyme in this incubated mixture at the hydrogen-ion concentration of the experiment and are not to be construed as indicating actual quantities of enzyme per cubic centimeter of tissue suspension.

LACTASE

Into a series of test tubes, 2 c c of tissue suspension, 2 c c of buffer solution of a desired hydrogen-ion concentration, and 5 c c of a 1 per cent solution of lactose were accurately measured, covered with a layer of toluene, and placed in a water bath at 37° C. Controls received the same quantity of boiled tissue suspension. After a certain period measured portions of the digest were removed and tested with Benedict's reagent to determine the extent of digestion. Table 2 shows that lactase was present in the mid-gut and active from pH 4.0 to 8.0, the optimum activity occurring from pH 6.0 to 8.0.

MALTASE

The procedure for determining the presence and activity of maltase was the same as that described under lactase, except that a 1 per cent maltose solution was used as substrate. Table 3 shows that maltase was present in the mid-gut. Its active range was found to be from pH 6.6 to 8.0, the optimum activity being at pH 7.6.

INVERTASE

The procedure for determining the presence and activity of invertase was as described under lactase, a 1 per cent solution of sucrose being used as substrate. As shown in Table 4, this enzyme was present in the mid-gut and was active throughout the pH range used (pH 4.0 to 8.0), being most active between pH 5.0 and 7.0, with optimum activity at pH 6.0.

TABLE 2.—*Hydrolysis of lactose by lactase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Extent of hydrolysis [a] at pH of—				
		4.0	5.0	6.0	7.0	8.0
		Mg	*Mg*	*Mg*	*Mg*	*Mg*
11	Normal	3.41	3.62	4.06	4.00	4.08
	Control	3.00	3.05	3.08	3.02	3.06
12	Normal	2.53	2.84	2.96	2.90	2.91
	Control	2.35	2.40	2.45	2.23	2.30
15	Fed Paris green	3.10	3.25	3.30	3.55	3.68
	Control	3.08	3.10	3.02	3.10	3.12
16	Fed Paris green	3.40	3.45	3.48	3.54	3.59
	Control	3.21	3.18	3.25	3.22	3.21
17	Fed lead arsenate	2.78	2.80	2.96	2.98	2.98
	Control	2.69	2.75	2.71	2.70	2.69
18	Fed lead arsenate	2.89	2.95	2.98	3.08	3.14
	Control	2.72	2.76	2.79	2.67	2.71

[a] In terms of milligrams of glucose per cubic centimeter of incubated mixture.

TABLE 3.—*Hydrolysis of maltose by maltase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Extent of hydrolysis [a] at pH of—						
		4.0	5.0	6.0	6.6	7.0	7.6	8.0
		Mg	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
23	Normal	2.30	2.30	2.30	2.50	2.55	2.70	2.50
	Control	2.20	2.18	2.20	2.19	2.21	2.16	2.20
24	Normal	2.20	2.23	2.28	2.46	2.50	2.80	2.68
	Control	2.12	2.18	2.14	2.10	2.11	2.13	2.10
27	Fed Paris green	3.17	3.20	3.33	3.38	3.48	3.59	3.36
	Control	3.12	3.15	3.09	3.14	3.18	3.17	3.13
28	Fed Paris green	3.39	3.27	3.41	3.39	3.56	3.67	3.40
	Control	3.20	3.25	3.24	3.20	3.27	3.29	3.19
29	Fed lead arsenate	2.81	2.89	2.88	2.97	2.94	2.90	2.98
	Control	2.79	2.76	2.80	2.69	2.82	2.78	2.80

[a] In terms of milligrams of glucose per cubic centimeter of incubated mixture.

TABLE 4.—*Hydrolysis of sucrose by invertase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Extent of hydrolysis [a] at pH of—				
		4.0	5.0	6.0	7.0	8.0
		Mg	*Mg*	*Mg*	*Mg*	*Mg*
32	Normal	0.55	2.00	2.35	1.00	0.55
33	do	.45	1.90	2.16	.93	.66
	Control	0	0	0	0	0
36	Fed Paris green	.79	2.21	2.48	.88	.65
37	do	.68	1.92	2.17	.73	.48
	Control	0	0	0	0	0
38	Fed lead arsenate	.85	2.46	2.59	.61	.58
39	do	.78	1.91	2.25	.79	.68
	Control	0	0	0	0	0

[a] In terms of milligrams of glucose per cubic centimeter of incubated mixture.

LIPASE

For determining the activity and presence of lipase, the titration of the free acid resulting from the hydrolysis of an olive-oil emulsion was used. In a 100 c c beaker, 25 c c of a 1 per cent neutral olive-oil emulsion and 12 c c of tissue suspension were placed, with thymol as a preservative. The controls contained the same quantity of boiled tissue suspension. The beakers were left in the dark at room temperature, and at intervals measured portions of the digest were removed and tested for acidity by titration with 0.05N sodium hydroxide. In Table 5 the acidity is indicated in cubic centimeters of 0.05N sodium hydroxide per 5 c c of the incubated mixture. These experiments demonstrated that lipase was present in the mid-gut.

PROTEOLYTIC ENZYMES

To follow the course of digestion by proteolytic enzymes several methods may be utilized. After preliminary work a 5 per cent gelatin solution, into which were incorporated several crystals of thymol as a preservative, was adopted as a substrate. The experiments covered a range of pH 2.0 to 8.0 with convenient pH intervals in order to test for the presence of both peptic and tryptic enzymes.

TABLE 5.—*Digestion of a neutral olive-oil emulsion by lipase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Acidity [a] after—						
		4 hours	8 hours	16 hours	24 hours	36 hours	48 hours	Total
		Cc	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
45	Normal	0.32	0.45	0.75	1.10	1.50	1.80	5.92
	Control	.15	.18	.20	.30	.35	.40	1.58
47	Fed Paris green	.37	.40	.65	.85	1.05	1.20	4.52
	Control	.11	.20	.25	.35	.35	.45	1.71
48	Fed lead arsenate	.30	.50	.70	.95	1.20	1.55	5.20
	Control	.15	.25	.30	.40	.40	.45	1.95

[a] In cubic centimeters of 0.05N sodium hydroxide per 5 cc of digest.

LIQUEFACTION OF GELATIN

Into each of a series of test tubes 10 c c of the gelatin solution previously adjusted to pH 7 with sodium carbonate, 2 c c of tissue suspension, and 2 c c of buffer solution of a desired hydrogen-ion concentration were accurately measured. A similar series containing boiled tissue suspension served as controls. The solutions were covered with a layer of toluene and were placed in a water bath at 37° C. At intervals the tubes were removed and immersed in an ice bath at 0° to 5° for 30 minutes, and the extent of digestion was estimated by observing the degree of liquefaction of the gelatin as described by Dernby (*5*), where the number 0 indicates no liquefaction and 6 indicates complete liquefaction. (Table 6.) The hydrogen-ion concentration of the contents of the tube reaching the highest number during the shortest interval of time indicated the optimum hydrogen-ion concentration at which digestion occurred.

The results of experiment 51 indicate two optima, one after 48 hours' digestion at pH 5, the other after 55 hours' digestion at pH 7. Although the pH range of activity in this case extended into both the acid and alkaline sides of neutrality—that is, from pH 4 to pH 8—it is quite evident, from what is known of the pH range at which pepsin is active, that in experiments 51 and 53 pepsin was not the enzyme concerned, for none of the contents of the tubes in the range of pH 2 to 4 gave any indication of digestion. The proteolytic enzymes present in the regurgitated liquid were active in the same range (pH 4 to 8), the optimum occurring in the shortest interval of time, namely, 24 hours at pH 5 to 8.

TABLE 6.—*Digestion of gelatin by proteolytic enzymes obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Age of tissue suspension	Period of digestion	Extent of liquefaction [a] at pH of—									
				2.0	2.6	3.0	4.0	5.0	6.0	6.6	7.0	7.6	8.0
		Days	*Hours*										
51	Normal	7	24	0	0	0	1	1	1	1	0	0	0
			48	0	0	0	1	6	5	2	4	0	0
			55	0	0	0	2	6	5	3	6	4	0
			72	0	0	0	2	6	6	5.5	6	6	5.5
	Control		72	0	0	0	0	0	0	0	0	0	0
53	Normal [b]	½	6	0	0	0	3	6	6	6	6	6	6
	Control		6	0	0	0	0	0	0	0	0	0	0
55	Normal	1	24	0	1	2	6	6	6	6	6	6	6
	Control		24	0	0	0	0	0	0	0	0	0	0
56	Normal	21	24	0	0	0	0	1	1	1	1.5	2	0
			48	0	0	0	0	1.5	3.5	4.5	5.5	5.5	5
	Control		48	0	0	0	0	0	0	0	0	0	0
66	Normal	2	24	0	0	0	1	4	5	5	6	6	5
			48	0	0	0	1	5.5	6	6	6	6	5.5
			72	0	0	0	1.5	6	6	6	6	6	6
	Control		72	0	0	0	0	0	0	0	0	0	0
60	Fed Paris green	1	24	0	1	3	5	6	6	6	6	6	6
	Control		24	0	0	0	0	0	0	0	0	0	0
69	Fed lead arsenate	2	24	0	0	0	0	0	0.5	2	3	4	5
			48	0	0	1	5.5	6	6	6	6	6	6
	Control		48	0	0	0	0	0	0	0	0	0	0
71	Fed lead arsenate	7	24	0	0	0	0	0	0	0	0	0	0
			48	0	0	0	0	0	0	1	2	2	0
			72	0	0	0	0	1.5	1.5	4	4.5	5.5	1.5
			96	0	0	0	0	2	4	4.5	5.5	6	4
	Control		96	0	0	0	0	0	0	0	0	0	0

[a] Complete liquefaction equals 6.
[b] Regurgitated liquid was used instead of tissue suspension.

The influence of the age of a tissue suspension on its activity was determined in the same manner as described above. A few typical results selected from the experiments are given in Table 6.

With a tissue suspension 1 day old (experiment 55) complete digestion took place in 24 hours at pH 4 to 8. With the same tissue suspension 2 days old (experiment 66) there was practically complete digestion after 48 hours at pH 5 to 8. With the same tissue suspension 1 week old (experiment 51) complete digestion occurred in 48 hours only at pH 5, but after 72 hours digestion was practically complete in every tube from pH 5 to pH 8. With the same tissue suspension 3 weeks old (experiment 56) practically complete digestion occurred only at pH 7 to pH 8 in 48 hours. In other experiments not shown in Table 6, performed with tissue suspensions 1 and 6 hours old, digestion in the tubes extended more and more into the acid range. Complete digestion at the pH range 2 to 4, however, was never attained. Hence the conclusion was drawn from these experiments that pepticlike enzymes are not present in the potato beetle.

Experiments were also performed with other proteins as substrates—namely, blood fibrin, casein, and albumin. All these experiments were quite conclusive in indicating that pepticlike enzymes are not present in the potato beetle.

FORMOL TITRATION METHOD

The digestion of gelatin by proteolytic enzymes was also tested by Sorensen's (*16*) formol titration method. This method depends on the formation of methylene compounds by the combination of formaldehyde with amino groups of amino acids. The acid carboxyl groups can then be estimated by titration with barium hydroxide or sodium hydroxide. The greater the concentration of the amino acids produced by the hydrolysis of a protein the greater the concentration of carboxyl and the greater the acidity following the addition of neutral formaldehyde solution. Thus the increased acidity is a measure of the degree of hydrolysis.

The formol titration was used in an experiment in which the hydrogen-ion concentration of the digest was adjusted at the beginning by the addition of hydrochloric acid or sodium hydroxide as was necessary. The change in acidity during digestion was measured in terms of cubic centimeters of 0.01N sodium hydroxide titrated to pH 8.4. A buffered solution of pH 8.4 was used as a standard, with phenolphthalein as an indicator. The effect of the addition of neutral formaldehyde solution after the titration of the free acidity was to render the solution again acid, and by titration the formol acidity was ascertained, as indicated in Table 7.

By selecting from this table data obtained during digestion at pH 5 and 7, which were the optima attained in the digestion of gelatin, it is noted that at pH 5 the free acidity rises progressively during the course of digestion, whereas the formol acidity does not. In the digestion at pH 7 the process is reversed, the free acidity remaining practically constant during the progress of digestion whereas the formol acidity rises considerably in the latter part of the period of digestion.

TABLE 7.—*Formol titration during digestion of gelatin by proteolytic enzymes of the potato beetle carried on at different hydrogen-ion concentrations*

pH	Free acidity [a] after—									Formol acidity [a] after—								
	4 hours	8 hours	14 hours	24 hours	30 hours	46 hours	52 hours	66 hours	Average	4 hours	8 hours	14 hours	24 hours	30 hours	46 hours	52 hours	66 hours	Average
	Cc	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
1.5	5.6	5.5	5.1	5.5	5.2	5.1	5.3	5.4	5.35	0.8	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.88
2.0	4.0	4.0	3.9	3.8	3.8	4.0	4.1	4.1	3.96	.8	.8	.9	.9	.8	.8	.8	.9	.84
2.6	3.9	3.9	3.7	3.7	3.5	3.5	3.8	3.8	3.73	.8	.9	.9	.9	.9	.8	.8	.9	.86
3.0	2.5	2.2	2.3	2.1	2.1	2.2	2.3	2.2	2.24	.8	.9	.9	.9	.9	.9	.9	.9	.89
4.0	1.8	1.8	1.8	1.9	1.8	1.8	1.9	1.9	1.84	.9	.9	1.0	.9	.9	1.0	1.0	1.0	.95
5.0	1.0	1.1	1.2	1.3	1.4	1.5	1.5	1.5	1.31	.9	1.0	1.0	1.0	1.0	1.1	1.2	1.2	1.05
6.0	.5	.6	.5	.6	.8	.7	.8	.8	.66	1.0	1.0	1.1	1.0	1.1	1.2	1.4	1.6	1.17
6.6	.5	.4	.5	.6	.5	.6	.7	.6	.55	1.0	.8	.9	1.0	1.0	1.0	1.2	1.2	1.01
7.0	.4	.5	.5	.5	.5	.5	.5	.6	.50	.9	1.0	1.0	1.0	1.1	1.2	2.0	2.1	1.29
7.6	.4	.2	.3	.4	.3	.4	.5	.7	.40	.9	1.0	.9	.8	.9	1.1	1.6	1.9	1.14
8.0	.3	.2	.2	.4	.4	.4	.3	.6	.35	.9	1.0	.9	1.0	.8	1.1	1.1	1.2	1.00

[a] Acidity expressed as cubic centimeters of 0.01N sodium hydroxide.

EREPSIN

Erepsin was determined by the method described by Swingle (*17*) and was found in the mid-gut and regurgitated liquid; none was found in the fore-gut or hind-gut. It is quite likely that the enzyme detected by this method does not differ from the one shown to be active in the liquefaction of gelatin.

THE EFFECT OF ARSENICALS ON THE ACTIVITY OF DIGESTIVE ENZYMES OF THE COLORADO POTATO BEETLE

METHODS OF INVESTIGATION

In the experiments to detect the effect of arsenicals on the activity of digestive enzymes, the insects were first placed on foliage previously sprayed with Paris green or lead arsenate in the proportion usually employed in field tests, namely, 2 pounds of the arsenical to 50 gallons of water. The insects selected for dissection were still alive and were taken from leaves that had been partly consumed. It is not definitely known whether all the insects used in the experiments had actually fed on poisoned foliage. However, it is surmised from their abnormal behavior that they had probably ingested some of the poison as they fed on the foliage. It is quite likely that the quantity of the poison consumed by different individuals varied considerably, and that some beetles may not have fed at all during these experiments, even when they were taken from leaves showing feeding areas. But the percentage of nonpoisoned beetles that may have been used in the dissections must have been very small indeed and would not have influenced the results to any great extent. Usually the beetles that fed on poisoned foliage ceased to feed and remained alive in some instances for several days. The dissection of such beetles revealed considerable food in the digestive tract. The food was removed, and the dissected parts were immersed in several changes of 0.2 per cent sodium chloride solution and distilled water before they were finally ground with sand and made into tissue suspensions.

Since the experiments with normal insects indicated that the enzymes were present only in the mid-gut, the preparation of tissue

suspensions for the following experiments was confined to the mid-gut. Two suspensions were prepared, a glycerol suspension and a water suspension, and the subsequent tests were carried out in the manner described under the treatment of the normal tissue suspension.

EXPERIMENTAL RESULTS

Amylase

The effect of arsenicals on the activity of amylase is shown in Table 1, experiments 5 and 6. In the case of beetles that fed on foliage sprayed with Paris green, a retardation of the initial activity of this enzyme was indicated in the range of pH 6.0 to 7.0, and complete digestion of starch was not attained even in 115 minutes. In the case of beetles that fed on foliage sprayed with lead arsenate, no retardation in the initial activity of this enzyme was observed, and, though complete digestion was not attained in 115 minutes, a higher degree of digestion than in the previous experiment was reached.

Lactase

Experiments 15 to 18 in Table 2 indicate no effect of arsenicals on the activity of lactase.

Maltase

The experiments with maltase, shown in Table 3, also disclose no influence of arsenicals on the activity of maltase.

Invertase

Table 4 demonstrates the activity of invertase of both the normal and the arsenic-fed beetles. Experiments 36 to 39 reveal no influence of arsenic on the activity of invertase.

Lipase

Experiments 47 and 48 in Table 5 disclose no definite influence of arsenicals on the activity of lipase.

Proteolytic Enzymes

The effect of arsenicals on the activity of proteolytic enzymes is shown in Table 6 by experiments 60, 69, and 71. Experiment 60 shows no influence of arsenic on the activity of tryptic enzymes when insects were fed foliage sprayed with Paris green. Similarly, experiment 69 shows no effect of lead arsenate on the activity of tryptic digestion; and experiment 71, in which the tissue suspension was 1 week old before testing, indicates no digestion whatever during the first 24 hours. Practically complete digestion occurred only in certain tubes after 96 hours, a condition similar to that recorded in the case of beetles that were not fed arsenicals, as shown by experiments 51 and 56.

In other experiments not indicated in Table 6, the beetles were injected with Paris green or lead arsenate suspensions. In both cases complete inhibition of the activity of the proteolytic enzymes occurred, no digestion being recorded after 5 days' incubation of the mixtures prepared. The greater inhibition of the activity of the proteolytic enzymes in the latter experiments was probably produced by the larger doses administered by injection.

DISCUSSION

The failure of moderate doses of Paris green and lead arsenate to inhibit markedly the activity of the digestive enzymes of the potato beetle is not surprising in view of the results reported in the literature (*1, 2, 3, 11, 12, 13, 14, 15*) on the effects of arsenicals on the activity of commercial enzymes. It seems to be generally agreed that small doses of arsenic acid, arsenious acid, atoxyl, ammonium arsenite, and methyl arsenoxide have no inhibiting effect on the activity of any of the enzymes investigated and in some instances even exert an activating influence. Stronger concentrations of arsenical solutions (from 2 to 10 per cent) caused retardation and complete inhibition of the activity of enzymes that attack disaccharides and proteins.

In the present experiments potato beetles were poisoned in the same way as in earlier experiments on the effect of arsenicals on the respiratory metabolism of insects (*6*). It can therefore be concluded that arsenicals exert a more profound effect on cellular respiration than on the activity of digestive enzymes of the potato beetle. Consequently the writer is inclined to believe that the toxic action of arsenicals on insects can not be explained, even in part, by their effect on digestive enzymes.

SUMMARY OF RESULTS

With few exceptions, in the normal insect all the enzymes studied, namely, amylase, lactase, invertase, lipase, and the proteolytic enzymes, were found only in the mid-gut and regurgitated liquid. In the exceptional cases it is believed that parts of tissue from the mid-gut may have adhered to the fore-gut and hind-gut during the process of dissection. This assumption is based on the fact that only a few experiments indicated digestion in either the fore-gut or hind-gut and that the digestive activity of the enzymes in these instances was extremely slight.

It is quite clear from experiments herein reported that arsenic does not influence the digestive enzymes of potato beetles fed on sprayed foliage to the extent of inhibiting their normal activity. In general, when they are fed on foliage sprayed with an arsenical, there is but little retardation of the activity of amylase and tryptic enzymes, and none at all of enzymes active in the digestion of disaccharides and fats. However, the injection of arsenical suspensions directly into the insect mouth results in complete inhibition of the activity of the proteolytic enzymes.

LITERATURE CITED

(1) Chittenden, R. H., and Allen, S. E.
1885. Influence of various inorganic and alkaloid salts on the proteolytic action of pepsin-hydrochloric acid. Conn. Acad. Arts and Sci. Trans. (1885–1888) 7:84–107.

(2) ——— and Cummins, G. W.
1885. Influence of various therapeutic and toxic substances on the proteolytic action of the pancreatic ferments. Conn. Acad. Arts and Sci. Trans. (1885–1888) 7:[108]–124.

(3) ——— and Painter, H. M.
1885. Influence of certain therapeutic and toxic agents on the amylolytic action of saliva. Conn. Acad. Arts and Sci. Trans. (1885–1888) 7:[60]–83.

142836—32——3

(4) Clark, W. M.
1922. The determination of hydrogen ions; an elementary treatise on the hydrogen electrode, indicator and supplementary methods, with an indexed bibliography on applications. Ed. 2, 480 p., illus. Baltimore.
(5) Dernby, K. G.
1918. A study of autolysis of animal tissues. Jour. Biol. Chem. 35:179–219, illus.
(6) Fink, D. E.
1926. Physiological studies of the effect of arsenicals on the respiratory metabolism of insects. Jour. Agr. Research 33:993–1007, illus.
(7) ———
1927. A micro method for estimating the relative distribution of glutathione in insects. Science (n. s.) 65:143–145.
(8) ———
1927. Is glutathione the arsenic receptor in insects? Jour. Econ. Ent. 20:794–801, illus.
(9) ———
1927. The application of studies in hydrogen ion concentration to entomological research. Ann. Ent. Soc. Amer. 20:503–512, illus.
(10) Parfentjev, J. A., and Devrient, W.
1930. Über die wirkung des arsens auf den gasstoffwechsel bei insekten. Biochem. Ztschr. 217:[368]–377.
(11) Pincussohn, L.
1908. Beeinflussung von fermenten durch kolloide. I. Wirkung von anorganischen kolloiden auf pepsin. Biochem. Ztschr. 8:[387]–398.
(12) Rona, P., Airila, Y., and Lasnitzki, A.
1922. Beiträge zum studium der giftwirkung. Über die kombinierte wirkung des chinins und der narkotica auf invertase und über die wirkung von arsenverbindungen auf maltase und α-methylglucosidase. Biochem. Ztschr. 130:[582]–591, illus.
(13) Schafer, F., and Böhm, R.
1872. Über den einfluss des arsens auf die wirkung der ungeformten fermente. Jahresber. Fortschr. Thierchemie 2:363–365.
(14) Smorodinzew, J. A., and Riabouschinsky, N. P.
1924. Zur frage nach dem einfluss von arsen- und antimonverbindungen auf die fermentativen funktionen des organismus. II. Mitteilung: Der einfluss einiger arsen- und antimonpräparate auf das pepsin. Biochem. Ztschr. 144:26–30.
(15) ——— and Riabouschinsky, N. P.
1926. Zur frage des einflusses von arsen- und antimonverbindungen auf die fermentativen funktionen des organismus. III. Mitteilung: Ändert sich die wasserstoffzahl des milieus während der verdauung des caseins durch pepsin in anwesenheit von arsen- und antimonverbindungen? Biochem. Ztschr. 168 : [73]–76.
(16) Sörensen, S. P. L.
1908. Enzymstudien. Biochem. Ztschr. 7:[45]–101.
(17) Swingle, H. S.
1925. Digestive enzymes of an insect. Ohio Jour. Sci. 25:209–218.
(18) Voegtlin, C., Dyer, H. A., and Leonard, C. S.
1925. On the specificity of the so-called arsenic receptor in the higher animals. Jour. Pharmacol. and Expt. Ther. 25:297–307.

A STUDY OF SEVERAL FACTORS IN THE SEPARATION OF SERUM FROM BOTTLED CREAM[1]

By G. MALCOLM TROUT, *Research Assistant in Dairying*, and J. C. MCCAN, *Graduate Research Assistant in Dairying, Michigan Agricultural Experiment Station*

INTRODUCTION

The separation of a layer of skim milk, or serum, at the bottom of the bottle of cream is a defect of considerable importance to the market-cream distributor. The consumer naturally attributes the presence of this skim milk, even when an insignificant layer appears in whipping cream containing as much as 40 per cent butterfat, to a very low fat content.

The greater portion of market cream is sold as table, or coffee, cream. Practically all of this grade of cream is standardized by the distributor to contain approximately 20 per cent butterfat. Any correlation by the consumer of the skim-milk layer with the fat content is especially undesirable because, as the distributor knows, many factors other than the fat content of the cream affect the volume of this serum layer.

The frequency of this cream defect and the attendant complaints from customers, combined with a lack of definite information bearing directly on the problem, seemed to make a study of some of the factors incident to this problem desirable.

REVIEW OF LITERATURE

A survey of the literature reveals many data on the creaming of milk, but although very closely associated, few data have been collected which bear directly on the separation of free serum from bottled cream. Many theories are current concerning the best methods of processing cream in the plant to reduce the prominence of this layer. Milk-plant practices in processing cream are based largely upon results obtained from cream-line studies of market milk and general observation in commercial plants.

The settling out of a layer of skim milk from bottled cream, or rather the "creaming off" of cream, seems to be the result of a greater degree of enmeshing and orientation of the individual fat globules and fat clusters. As early as 1899 Farrington and Russell (*2*)[2] observed that the cream volume on milk was reduced after the milk had been creamed for 48 hours. The reduction of the cream volume of milk following its initial formation was later observed by Dahlberg and Marquardt (*1*), Hammer (*3*), Judkin and Downs (*4*), Martin and Combs (*5*), Trout (*6*), and Whitaker et al. (*8*).

The fat content of gravity cream has been observed (*6*) to range from 11.0 to 28.5 per cent, depending upon the nature and previous treatment of the milk and upon the conditions of creaming. Following the introduction of improved machinery and new methods, the

[1] Received for publication Jan. 26, 1932; issued October, 1932. Journal article No. 87 (n. s.) from the Michigan Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 500.

standardization of table cream to approximately 20 per cent butterfat became the common practice of market-cream distributors. This seemingly low fat content of the standardized cream in comparison with that of some gravity cream secured under certain conditions has had its part in causing the cream distributor to homogenize his product, although the principal reason perhaps for homogenizing was to increase the viscosity of the cream, which gave it a heavier body and thus a richer appearance. However, beyond certain pressures of homogenization, as pointed out by Webb and Holm (7), feathering of the cream resulted when homogenized cream was added to coffee. The procedure in many smaller milk plants in processing market table cream is that only of standardizing the fat content of the cream, and the homogenization process is entirely eliminated. This cream, when bottled and held at storage temperatures ranging from 35° to 40° F. for 24 to 48 hours, often revealed a serum layer, which was objectionable to the consumer for the reasons previously mentioned.

SCOPE OF THE INVESTIGATION

Few data are available that show the effect of the temperature at which the milk is skimmed upon the separation of free serum from the resulting cream, either before or after standardization. Likewise, there is little information regarding the effect of the heat treatment of milk previous to separation on the appearance of the serum layer. Practically all the effects noted as resulting from certain milk-plant operations have been concerned with the creaming of milk rather than with the stability of the cream once it has formed. The object of this study was to determine the effects on the stability of cream of various factors, such as heat treatment of milk and cream, standardization, viscolization, and the addition of gelatin and salts.

METHODS

Cream was obtained from mixed milk containing from 3.4 to 3.8 per cent butterfat. This milk was secured each day from the same 300-gallon pasteurizer in the college creamery after it had been filled with milk delivered by the same group of milk patrons.

For the major part of the experimental work, for which only small lots of cream were required, the milk was separated in the experimental laboratory with a small electrically driven separator. Preliminary runs were made to determine the proper screw setting to yield, at the various temperatures, cream having approximately the desired percentage of butterfat. One gallon of milk was allowed to run through the machine in order to temper the bowl before the samples of cream and skim milk were saved. Larger quantities of cream were obtained by separating milk with a larger separator in the college creamery. In one series of experiments, so far as possible, a 20 per cent cream was separated, while in another series approximately a 40 per cent cream was secured, which was later standardized back to 20 per cent butterfat by being combined with the skim milk previously separated.

The milk, both fresh and stored, was skimmed at temperatures extending from 60° to 145° F. before pasteurization, at 145° for 30 minutes, and at similar temperatures following the pasteurization exposure. One series of experiments was conducted in which ex-

posures prior to separation of 145° for 30 minutes, 160° for 15 minutes, and 180° momentarily following the 160° exposure were used.

Four lots of raw cream which had been separated at 90° to 110° F. and standardized to 20 per cent butterfat were exposed for 30 minutes to temperatures of 145°, 150°, 155°, and 160°, respectively. Similarly standardized cream secured from pasteurized milk was exposed to the same degree of temperature for the same length of time. The holding or vat method of pasteurization was used.

Some of the cooling was done in the pasteurizing vats, some by placing the cream or milk in 1-gallon cans set in water and gently stirred at intervals, and some over a surface cooler. The product was cooled to 60° F. for standardization or for taking the samples.

When trials were made with gelatin a high grade of commercial gelatin was used. The gelatin was mixed with 1 pint of cold water and heated in a tank of hot water until completely dissolved. The dissolved gelatin was then added to a 60-pound lot of milk in the pasteurizer at 120° F. as the milk was being heated to 145° for separation.

Various percentages of sodium carbonate, calcium chloride, monocalcium phosphate, dicalcium phosphate, and sodium citrate were added to the cream. With the exception of dicalcium phosphate, which was not so soluble, these salts were made up so that 5 c c of the solution would give 0.1 per cent of the salt when added to a 500 c c sample of cream. Dicalcium phosphate was added as a saturated solution.

The condensed milk used was a well-known commercial brand of sterilized unsweetened evaporated milk which had been concentrated approximately in the ratio of 2 to 1. The milk powder used, testing 95 per cent solids, was made by the spray process and was obtained from a local manufacturer. The casein used was a commercial brand of soluble food casein.

Samples of the cream were taken in order to determine the volume of the serum layer appearing in the cream, and also to observe the distinctness or attention-attracting power of this layer, as evidenced (1) by the sharpness of the line of demarcation between the layers, and (2) by the difference in the appearance of the cream and the serum layers as a result of the completeness of separation of the fat. It was believed at first that both observations could be made by setting the cream in standard 100 c c graduated cylinders which were used to determine the volume of the layer of free serum. Preliminary runs, however, showed that the distinctness of the serum layer was always much greater in bottles than in the cylinders. Samples were, therefore, taken both in cylinders and in ½-pint cream bottles; in the cylinders to determine the volume of the serum layer and in the bottles to observe the distinctness of this layer. The samples were taken after the cream had been cooled to approximately 60° F. The cylinders and bottles were then cooled in ice water to 40° and set aside for creaming.

The milk and cream were stored in the cold-storage room of the creamery, which ranged in temperature from 35° to 40° F. All samples set for creaming were placed in this room, with the exception of a few samples in that part of the experiment concerned with the effect of the temperature of creaming. In this study samples were set in ice water in the cold room at 32°, in an electric refrigerator held at a constant temperature between 39° and 41°, in cold running water main-

tained at 56°, and in a culture oven kept at 68° to 70°. Two drops of 40 per cent formaldehyde were added to each pint of cream as a preservative, after preliminary study had showed that the addition of this preservative in considerably larger quantities had no noticeable effect on cream rising.

All butterfat tests were made according to the Babcock method.

The observations of creaming were made after 48 hours, except when it was necessary to study the results after 72 hours of creaming. Considerable difficulty was encountered in this part of the work, especially in observing the creaming in the cylinders, where the separation of the layers was far less distinct than in the bottles. Sudan III was used in an attempt to make more accurate and more reliable readings possible but without success. A microscope light was tried in order to standardize the source of light for these observations. Preliminary work, however, showed that the best source of light was indirect sunlight, which was used in making all observations.

The volume of the serum layer was recorded in terms of percentage. The degree of distinctness of the skim-milk layer was recorded by means of letters, as follows: A = wholly distinct; B = very distinct; C = fairly distinct; D = not very distinct; E = barely observable; F = no layer, but showing a different appearance between the lower and upper portions due to some separation of skim milk; and G = no evident separation.

The results of the work recorded represent averages in the majority of cases of at least six trials of every factor studied; in some cases the results of only four trials, while in others the averages of many more trials are recorded. A total of 126 different lots of cream was prepared, treated, and observed.

EFFECT OF THE HEAT TREATMENT OF THE MILK PRIOR TO SEPARATION

FRESH MILK

The temperature of the fresh milk at the time of separation was found to influence both the amount and the distinctness of the skim-milk layer, as shown in Tables 1 and 2. Separation of the milk at the receiving temperature, 55° to 65° F., gave a deeper serum layer than did separation at any of the higher temperatures. This difference was very evident in the bottled cream as well as in the cylinders. Temperatures of separation ranging from 90° to 180° were found to have little influence on the amount of the serum; although there was considerable difference in the distinctness of the layer. Separation at the receiving temperature, 55° to 65°, or at 90°, gave a considerably more distinct serum layer than did separation at 120° or above. At 145° the layer was more distinct than at 120°. This difference, however, was only slight and might have been due to the effect of heat on the milk during the time required for the separation process, since pasteurization at 145° for 30 minutes, as will be shown in the consideration of the effect of pasteurization, was found to increase the distinctness of the layer.

TABLE 1.—*Effect of heat treatment of fresh milk, prior to separation, on the volume and distinctness of the serum layer when low-test cream was secured*

[Average of six trials]

Lot No.	Heat treatment	Fat in cream	Serum layer	Distinctness of serum layer
		Per cent	*Per cent*	
1	Heated to 90° F. (check)	20.8	1.83	B−
2	Heated to 120° F	19.5	2.04	D
3	Heated to 145° F	20.6	2.0	C−
4	Heated to 145° F. for 30 minutes, then cooled to 120°	21.2	2.1	C+
5	Heated to 145° F. for 30 minutes, then cooled to 90°	22.0	2.0	C+

TABLE 2.—*Effect of heat treatment of fresh milk, prior to separation, on the volume and distinctness of the serum layer, when high-test cream was secured and standardized back to approximately 20 per cent butterfat*

[Average of six trials]

Lot No.	Temperature of separation	Fat in cream secured	Fat in standardized cream	Serum layer	Distinctness of serum layer
		Per cent	*Per cent*	*Per cent*	
1	55–65° F	50.4	20.4	2.83	B
2	90° F	38.5	19.9	2.2	B+
3	120° F	42.3	20.1	2.38	D−
4	145° F	41.8	20.6	2.0	D
5	145° for 30 minutes	44.4	20.2	2.0	C−

The influence of the temperature of separation at 90° and 120° F. varied, depending upon whether the milk was being heated up to those temperatures or was being cooled from a higher temperature. Pasteurizing milk at 145° for 30 minutes, followed by subsequent cooling to 120° and 90° for separation, gave no appreciable difference in the distinctness or volume of the serum layer at the two temperatures. However, when the temperature of the milk was raised from the temperature at which it was received at the plant to 90° and to 120°, separation at 90° gave a far more distinct cream line.

STORED MILK

The results of storing milk for 24 to 48 hours at 35° to 40° F. and separating the cream after slowly and carefully warming the milk to the various temperatures are given in Table 3. The temperature of separation of old milk was found to have little effect on the amount of the serum layer. The results indicate that the time of storing the milk at low temperatures had little influence upon the volume of the serum layer as compared to that obtained from fresh milk, except at 60°. A comparison of the data in Tables 2 and 3 shows that when old stored milk is separated at that temperature the serum layer is reduced to 2.07 per cent, whereas in the case of fresh milk it is 2.83 per cent. The distinctness of the serum layers varied somewhat according to the temperature of separation.

When the temperature of the old stored milk was raised to 80° to 90° F. before separation the cream yielded showed a much more distinct serum layer than when the separating was done at 60°. Separating old milk at 80°, 90°, 120°, and 145° revealed that there was little

difference between separating old milk and separating fresh milk at similar temperatures in regard to the distinctness of the serum layer. As with separating fresh milk, separating old milk at 120° gave a much less distinct layer than separating it at 80° or 90°. Raising the temperature from 120° to 145° before separating increased the distinctness to a slight extent.

TABLE 3.—*Effect of heat treatment of stored milk, prior to separation, on the volume and distinctness of the serum layer*

[Average of eight trials]

Lot No.	Temperature of separation	Fat in cream secured	Fat in standardized cream	Serum layer	Distinctness of serum layer
		Per cent	*Per cent*	*Per cent*	
1	60° F.	24.5	20.5	2.07	C
2	80° F.	20.2	20.2	1.95	A−
3	90° F.	20.4	19.8	2.14	B+
4	120° F.	19.3	19.3	1.84	E+
5	145° F.	20.3	20.3	2.0	D+

PASTEURIZATION

The results of separating milk after heating it to 145° F. momentarily, after pasteurizing it at 145° for 30 minutes, after pasteurizing it at 145° and cooling it to 120°, after pasteurizing it at 160° for 15 minutes, and after raising the temperature of the milk pasteurized at 160° to 180° indicate (Table 4) that, with the exception of the milk pasteurized at 160° for 15 minutes, the pasteurization exposure had little influence upon the amount of the serum layer. When the milk was pasteurized at 160° for 15 minutes and skimmed at that temperature, a smaller volume of serum appeared in the resulting cream than appeared in that secured at any other temperature of pasteurization. These results are shown in Tables 1, 2, and 4.

TABLE 4.—*Effect of high temperature of fresh milk, prior to separation, on the volume and distinctness of the serum layer*

[Average of seven trials]

Lot No.	Heat treatment	Fat in cream	Serum layer	Distinctness of serum layer
		Per cent	*Per cent*	
1	Heated to 145° F. (check)	19.8	2.13	D
2	Heated to 160° F. for 15 minutes	20.4	1.76	C−
3	Heated to 160° F. for 15 minutes, then raised to 180° for separation	20.7	2.11	C−

Heating milk to 145° F. and separating it immediately resulted in a less distinct serum layer than was obtained in the check sample secured at 90°. Pasteurizing the milk at 145° for 30 minutes made the serum layer more distinct than that secured when the milk was merely heated to 145°, but the layer was considerably less distinct than that secured in the check sample at 90°. Pasteurizing the milk at 160° for 15 minutes, or at this same exposure and then raising the temperature to 180° momentarily, before separating, gave a slightly

more distinct serum layer than was obtained when the milk was heated only to 145° momentarily. (Table 4.)

EFFECT OF HEAT TREATMENT OF THE CREAM

The results obtained by pasteurizing raw standardized cream and repasteurizing pasteurized standardized cream are given in Table 5. Pasteurizing raw cream at each of the 5-degree intervals from 145° to 160° F. for 30 minutes yielded a serum layer of less volume but of greater distinctness than that in the raw cream. The different temperatures used between 145° and 160° apparently had no marked influence either on the volume or on the distinctness of the layer. One temperature was as satisfactory as the other. Approximately a 1.9 per cent serum layer appeared in the cream obtained from the pasteurized cream, as compared with a 2.5 per cent serum layer in the cream obtained from the raw cream.

TABLE 5.—*Effect of different temperatures of pasteurization of raw and pasteurized standardized cream on the volume and distinctness of the serum layer*

[Average of four trials]

Temperature maintained for 30 minutes	Lots in which cream and skim milk used for standardization were obtained from raw milk				Lots in which cream and skim milk used for standardization were obtained from milk pasteurized at 145° F. for 30 minutes			
	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
	Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*	
Unpasteurized (check)	47.5	20.0	2.5	D−	38.8	20.0	1.85	B−
145° F.	47.5	20.0	1.95	C+	38.8	20.0	1.95	B−
150° F.	47.5	20.0	1.98	C+				
155° F.	47.5	20.0	1.9	C+				
160° F.	47.5	20.0	1.85	C+	38.8	20.0	1.8	B−

Repasteurizing standardized pasteurized cream at 145° F., or at 160°, for 30 minutes apparently had no appreciable effect either on the volume or on the distinctness of the serum layer, as shown by the results in Table 5. This cream was secured by mixing pasteurized whole milk with cream from pasteurized milk.

EFFECT OF VARIATIONS OF THE MANY FACTORS CLOSELY ASSOCIATED WITH STANDARDIZATION

HIGH-TEMPERATURE PASTEURIZATION OF THE MILK VERSUS HIGH-TEMPERATURE PASTEURIZATION OF THE CREAM USED IN STANDARDIZATION

Lots of raw cream testing 38.5 per cent butterfat were standardized with raw skim milk and with skim milk pasteurized at 170° F. for 20 minutes. Also lots of raw skim milk were used to standardize raw high-fat-content cream and similar cream pasteurized at 170° for 20 minutes.

TABLE 6.—*Effect of high temperature of pasteurization of either the skim milk or cream, or both, before and after standardization*

[Average of six trials]

Product and heat treatment [a]	Lots in which cream and skim milk used for standardization were obtained from raw milk				Lots in which cream and skim milk used for standardization were obtained from milk pasteurized at 145° F. for 30 minutes			
	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
	Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*	
Cream pasteurized before standardization	38.5	20.7	2.3	C	44.6	20.0	2.47	D
Skim milk pasteurized before standardization	[b] 38.5	[b] 20.7	2.45	·B	[b] 44.6	[b] 20.0	2.20	D
Cream pasteurized after standardization	38.5	19.8	4.3	C−	44.6	20.25	6.17	D−

[a] 170° F. for 20 minutes. [b] Percentage of fat in the cream.

The data presented in Table 6 show little difference in the volume of the serum layers appearing in either of the standardized creams of which one or the other of the products used was heated to a high temperature. It will be observed that the detrimental effect was very great when the cream was pasteurized at 170° F. for 20 minutes after standardization. Similar results were obtained when the cream and skim milk were secured from pasteurized milk rather than from raw milk.

When the skim milk was secured from raw whole milk and was pasteurized before being used in standardizing the raw cream the distinctness of the serum layer was much greater.

STANDARDIZING PASTEURIZED CREAM WITH MILK PROCESSED IN VARIOUS WAYS

High-test cream was standardized with raw whole milk, with pasteurized whole milk, and with skim milk from pasteurized milk. The normal exposure of 145° F. for 30 minutes was employed. It will be observed from the data in Table 7 that no appreciable differences occurred in the volume of the serum layer from the cream standardized with the different milks, either raw or pasteurized. The distinctness of the layer, however, was very slightly less in every case when raw whole milk was used in the standardization than when pasteurized whole milk or pasteurized skim milk was used.

TABLE 7.—*Effect of standardizing pasteurized cream with raw whole milk, with pasteurized whole milk, and with skim milk from pasteurized milk*

[Average of five trials]

Lot No.	Kind of milk used	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
		Per cent	*Per cent*	*Per cent*	
1	Raw whole	45.2	20.7	1.88	C−
2	Pasteurized whole	45.2	20.3	1.98	C+
3	Skim milk from pasteurized milk	45.2	20.4	2.08	C+

SEPARATING HIGH-TEST CREAM AND STANDARDIZING BACK VERSUS SEPARATING LOW-TEST CREAM

The opinion seems to be current among milk-plant operators that high-test cream standardized back to a low fat content gives a greater serum layer than cream separated to contain a low fat content, which requires less standardizing. The data obtained in this experiment as presented in Table 8 show a tendency toward a higher volume of serum layers in the high-test cream standardized back to approximately 20 per cent than in the cream separated as near 20 per cent as possible and not standardized. However, with many difficult readings due to the indistinctness of the serum layers in the cylinders in this experiment, definite conclusions can not be drawn. Apparently there was no significant difference in the distinctness of the serum layer in the standardized and unstandardized cream.

TABLE 8.—*Effect of standardizing high-fat-content cream back to approximately 20 per cent butterfat with the corresponding skim milk obtained during the separation as compared with results on a 20 per cent cream as separated*

[Average of six trials]

Temperature of separation	Cream standardized back to about 20 per cent fat content				Cream of about 20 per cent fat content as separated			
	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer	Fat before standardization	Fat after standardization	Serum layer	Distinctness
	Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*	
90° F	38.5	19.9	2.2	B+	20.8	20.8	1.83	B−
120° F	42.3	20.1	2.38	D−	19.5	19.5	2.04	D
145° F	41.8	20.6	2.0	D	20.6	20.6	2.0	C−

STANDARDIZING WITH WHOLE MILK VERSUS STANDARDIZING WITH SKIM MILK

The data secured when raw cream was standardized with raw whole or with raw skim milk presented in Table 9, show little difference either in the amount of the serum layer or in its distinctness due to the factor of richness of the standardizing media. On the other hand, when pasteurized cream was standardized with pasteurized whole milk or with pasteurized skim milk, a serum layer of less volume appeared in the cream (Table 7) than when raw cream was standardized with raw whole milk or with raw skim milk (Table 9). This appears to substantiate the former results obtained showing that pasteurization decreased the serum layer.

TABLE 9.—*Effect of standardizing raw cream with raw whole milk versus standardizing raw cream with raw skim milk*

[Average of six trials]

Milk used	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
	Per cent	*Per cent*	*Per cent*	
Whole	46.0	20.3	2.3	C
Skim	46.0	20.8	2.5	D

STANDARDIZATION BEFORE VERSUS STANDARDIZATION AFTER PASTEURIZATION

In studying the effect of standardization before as compared with standardization after pasteurization, raw cream was standardized with raw whole milk. This product was then pasteurized at the ordinary exposures. The results were compared with those obtained by standardizing previously pasteurized high-test cream with pasteurized milk. It will be observed from the data shown in Table 10 that the serum layer was somewhat greater in volume when the standardizing was done prior to pasteurization than when it was carried out after pasteurization. There seemed to be no marked difference in distinctness as a result of the two methods.

TABLE 10.—*Effect of standardizing cream before versus standardizing it after pasteurization*

[Average of five trials]

When standardized	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
	Per cent	*Per cent*	*Per cent*	
Before pasteurization	45.2	20.4	1.9	B−
After pasteurization	42.2	20.9	1.5	B−

TEMPERATURE OF STANDARDIZATION

Since the temperature of separation and pasteurization found to affect the distinctness and volume of the serum layer, it was thought possible that the temperature of the milk and of the cream at the time of mixing in standardization might have an influence on the volume and distinctness of the serum layer. However, the results of four trials, in which the milk and cream were mixed hot and cold in all possible combinations, showed that neither the temperature of the cream nor that of the milk had any appreciable effect on the amount or on the distinctness of the serum layer when raw cream was standardized with raw skim milk.

STANDARDIZING CREAM FRESH VERSUS STANDARDIZING IT AFTER 24 HOURS IN STORAGE

The data secured indicate that a slightly greater serum layer formed when the cream was standardized fresh. However, standardization after storing gave a layer of considerably greater distinctness, as will be observed from the data presented in Table 11.

TABLE 11.—*Effect of standardizing cream fresh versus standardizing cream after storing 24 hours*

[Average of seven trials]

Kind of cream standardized	Fat before standardization	Fat after standardization	Serum layer after—		Distinctness of serum layer after—	
			48 hours	120 hours	48 hours	120 hours
	Per cent	*Per cent*	*Per cent*	*Per cent*		
Fresh	42.7	19.8	1.91	4.29	C−	E+
Stored 24 hours at 35° F.	42.7	19.9	1.75	3.71	B−	C−

EFFECT OF MISCELLANEOUS CONDITIONS

AGITATION OF CREAM AFTER STORING

The cream was stored in the cold-storage room at 35° to 40° F. for 24 hours, after which it was agitated violently, to determine whether such agitation, after the fat was in a hardened condition, had any influence on the serum layer. The data secured are presented in Table 12. It was found that agitation of pasteurized cream after it had been stored 24 hours very slightly increased the volume of the serum layer. Furthermore, agitation of the stored cream caused the formation of a more distinct layer.

TABLE 12.—*Effect of agitating standardized cream which had been stored 24 hours at a low temperature*

[Average of six trials]

Agitation of cream	Temperature of separation	Temperature of pasteurization	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
	° F.	*° F.*	*Per cent*	*Per cent*	*Per cent*	
Unagitated (check)	90–100	145	44.4	20.1	2.1	C–
Agitated after 24 hours at 35° F	90–100	145	44.4	20.1	2.2	B–

METHOD OF COOLING

Since considerable data are found in the literature showing greater creaming when the milk is cooled rapidly, a study of this factor was made with pasteurized cream to determine the degree of separation of free serum. The rapid cooling was done over a surface cooler, while the slow cooling was accomplished by occasionally gently stirring the cream, which was set in a vat of cold water. The data obtained, as presented in Table 13, indicate that the speed of cooling pasteurized cream had little effect either upon the volume or upon the distinctness of the resulting serum layer.

TABLE 13.—*Effect of the method of standardizing and cooling cream*

When standardized	Trials	Temperature of separation	Fat before standardization	Fat after standardization	Serum layer when—		Distinctness of serum layer when—	
					Surface cooled	Vat cooled	Surface cooled	Vat cooled
	Number	*° F.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
Before pasteurization	5	90–100	45.2	20.4	1.88	1.94	B–	C–
After pasteurization	5	90–100	45.2	20.9	1.66	1.64	C+	C
After cooling following pasteurization	4	90–100	44.9	20.5	1.90	1.83	C	C–

TEMPERATURE OF CREAMING

The data presented in Table 14 show no appreciable difference either in the volume or in the distinctness of the serum layer when the creaming was attempted in ice water as compared with that secured at the cooling-room temperatures of 35° to 40° F. Later work, after an accurate, temperature-controlled, electric refrigerator had been obtained and the higher temperature constantly main-

tained at 39° to 41°, gave data which seemed to indicate that at ice-water temperature a slightly smaller serum layer was formed than formed at 39° to 41°. At higher temperatures the layers were even less distinct. The actual volume of the layers could not be observed in the cylinders as the line of demarcation was indistinct, but the volume of the serum layers in the bottles was larger at 56° and at 70°, the volume at the latter temperature being the greater.

TABLE 14.—*Effect of the temperature of creaming*

Temperature of separation	Trials	Fat before standardization	Fat after standardization	Serum layer when creaming was done in or at—					Distinctness of serum layer when creaming was done in or at—				
				Ice water	35°–40° F.	39°–41° F.	56° F.	70° F.	Ice water	35°–40° F.	39°–41° F.	56° F.	70° F.
	Number	*Per cent*	*Per cent*										
90° to 100° F.	4	45.5	20.5	1.83	1.8	.....	(a)	(a)	C	C	.....	D–	F+
145° F.	5	36.0	20.0	1.86	.....	2.08	.....	.....	B	.....	B–	.....	.....

[a] Too indistinct for accurate reading.

EFFECT OF DURATION OF TIME OF CREAMING

The data secured indicate that as the duration of time of creaming was extended the volume of the serum layer increased. The distinctness of the serum layer increased for the first 72 hours; after that time its distinctness diminished. It was difficult to determine the volume of the serum layer, especially after 96 hours, as the serum layers in the cylinders became less distinct. The distinctness seemed to increase for some 48 to 72 hours and then gradually decreased. Many observations in the study of the various factors were made at the end of 96 hours, in addition to the observations at the end of 48 hours, as a check on the first readings. It was always very evident that the layer was less distinct after 96 hours. In studying the effect of storing the milk and cream a day before standardization, in which a comparison of the stored, standardized sample was made with the check sample set raw on the fifth and sixth days of creaming, respectively, it was found in every case that the serum layers were considerably less distinct after creaming had been going on for five or six days than after creaming had been going on only 48 hours. The serum layers were slightly more than twice as great in volume after the longer period of creaming. (Fig. 1.)

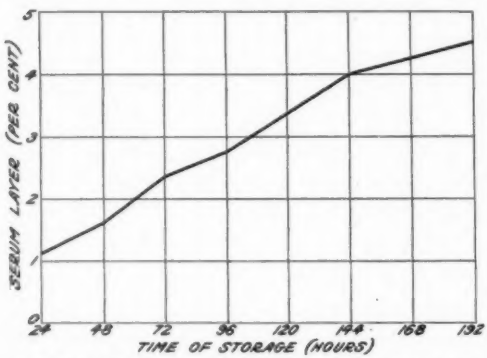

FIGURE 1.—Increase of serum layer formed in cream during storage at 37° F.

EFFECT OF VISCOLIZATION

Temperature and Pressure

Raw standardized cream was viscolized at 145° F. and at 160° at pressures of 500, 1,000, 1,500, 2,000, and 2,500 pounds per square inch. The data presented in Table 15 show that low pressures reduced the creaming as compared with the unviscolized cream, and that the volume of the serum layer was reduced by increases in pressure until the layer was entirely eliminated. Since free serum was sometimes noted in the bottles when there was no apparent separation of the cream in the cylinders this was tabulated in the results. A "small layer" indicates that an appreciable amount of separation took place and would probably be noticed by the cream buyer. A "trace" indicates a very small layer often noticed only after careful observation of the samples, and would probably never be evident to the consumer. It was found that at low pressures at which the serum layer was not entirely eliminated, the small remaining layer was more distinct than in the check, the unviscolized samples. Viscolizing at 160° as compared with viscolizing at 145° generally gave a less distinct serum layer at low pressures, when a serum layer appeared. The layer was also usually entirely eliminated at a lower pressure when the milk was processed at 160° rather than at 145°, thus indicating that viscolization was more efficient at high than at low temperatures. Viscolizing raw cream at 1,500 pounds pressure per square inch at 145° or 1,000 pounds pressure at 160° ordinarily eliminated the serum layer entirely or reduced the layer to such an extent that it would probably have passed unnoticed by the consumer.

Table 15.—*Effect of viscolizing raw cream originally containing 45.88 per cent fat but standardized at 20 per cent at various pressures and temperatures*

[Average of four trials]

Pressure used in viscolizing (pounds per square inch)	Serum layer when cream was processed at 145° F.		Distinctness of serum layer when cream was processed at 145° F.		Serum layer when cream was processed at 160° F.		Distinctness of serum layer when cream was processed at 160° F.	
	Unviscolized	Viscolized	Unviscolized	Viscolized	Unviscolized	Viscolized	Unviscolized	Viscolized
0	*Per cent* 2.1	*Per cent*	C−		*Per cent* 1.75	*Per cent*	C−	
500		(a)		C−		(a)		E−
1,000		(b)		C−		(a)		F
1,500		(a)		C−		None.		G
2,000		None.		F		None.		G
2,500		None.		G		None.		G

[a] Too indistinct for accurate reading. [b] Small layer.

Viscolizing High-Test Cream

Attempts to eliminate the serum layer by viscolizing high-test cream before standardizing it with unviscolized milk proved unsuccessful. However, the serum layer was always considerably reduced in volume by this treatment. On the other hand, this method gave a much more distinct serum layer, thus overcoming the advantages of the reduced volume.

Viscolizing Only a Portion of the Cream

By mixing 10 per cent of cream, standardized to 20 per cent fat and viscolized at 2,500 pounds pressure with unviscolized cream testing 20 per cent fat, the volume of the serum layer was decreased from 2.1 to 1.18 per cent, or reduced approximately 44 per cent. The treatment had no significant influence on the distinctness of the layer as compared with that in the check samples which had not been viscolized.

EFFECT OF THE ADDITION OF VARIOUS SALTS

Sodium carbonate, sodium citrate, calcium chloride, dicalcium phosphate, and monocalcium phosphate were added in varying percentages to cream in order to determine the effect of these salts on the separation of free serum. The cream used was separated from milk at temperatures between 90° and 100° F., was standardized from 44.5 per cent butterfat to 20 per cent butterfat, and was pasteurized at 145° for 30 minutes. After the salts were added, the samples were held at 35° to 40° for 48 hours before observations were made. The results are given in Table 16. The addition of 0.1 per cent sodium carbonate considerably increased the volume and made the layer slightly more distinct than in the check samples. The addition of 0.3 and 0.5 per cent decreased the volume as compared with that when 0.1 per cent was used, but a partial precipitation of the curd took place, as demonstrated by the clear whey which was left in the bottom instead of the usual serum.

Table 16.—*Effect of the addition of various salts to pasteurized cream containing 20 per cent of butterfat*

[Four trials in each test]

SALT ADDED BY WEIGHT, DRY

Salts used	Serum layer when indicated percentage of salt was added				Distinctness of serum layer when indicated percentage of salt was added			
	None	0.1	0.3	0.5	None	0.1	0.3	0.5
Sodium carbonate	1.78	3.6	2.28	1.95	C−	B−	A+	A+
Calcium chloride	1.78	1.75	1.75	1.75	C−	C−	D	D−
Dicalcium phosphate	1.78	1.95	1.85	1.88	C−	C−	C−	C−
Sodium citrate	1.78	1.65	3.33	3.45	C−	C−	A+	A+

SALT ADDED AS CUBIC CENTIMETERS OF SATURATED SOLUTION

	None	5	15	25	None	5	15	25
Monocalcium phosphate	1.78	2.33	2.25	8.25	C−	C−	D	D

CUBIC CENTIMETERS OF WATER ADDED, CHECKS

	None	5	15	25	None	5	15	25
Check samples	1.78	1.75	1.85	1.93	C−	C−	C−	C−

The addition of calcium chloride in the various amounts apparently had no marked influence on the volume of the serum layer, but the addition of 0.3 and 0.5 per cent resulted in the serum layer becoming less distinct.

Dicalcium phosphate in the amounts added apparently had no measurable effect either on the size or on the distinctness of the serum layer.

The addition of one-tenth of 1 per cent sodium citrate evidently had no marked effect either on the volume or on the distinctness of the layer, but adding 0.3 and 0.5 per cent markedly increased both the volume and the distinctness of the layer. This may have been due to a partial precipitation of the casein, for the serum was only slightly milky at the bottom. This indicated that the greater portion of the casein had been carried up with the fat.

The addition of 5, 15, and 25 c c of a saturated monocalcium phosphate solution increased the volume of the serum, especially in case of the most concentrated sample. This salt also reduced the distinctness of the layer to a slight extent, particularly when 15 c c and 25 c c additions of the solution were made. The 15 c c concentration caused a slight precipitation of the casein and the 25 c c concentration caused a considerable precipitation which was noticed during the agitation of the samples at the time the solution was added.

EFFECT OF THE ADDITION OF GELATIN, EVAPORATED MILK, SKIM-MILK POWDER, AND SOLUBLE FOOD CASEIN

Instead of being added to the cream, the gelatin was added to the milk prior to separation. The cream secured from the various lots was then compared after being stored at 35° to 40° F. for 30 minutes. Except when gelatin was added, the cream used in these experiments was separated from milk at 90° to 100°, then standardized from 45 per cent butterfat to 20.5 per cent butterfat. Before the addition of the various substances, the standardized cream was pasteurized at 145° for 30 minutes. After the substances had been introduced and mixed the samples were stored at 35° to 40° for 48 hours before observations were made.

GELATIN

Some very interesting results were obtained by adding small amounts of gelatin to the milk prior to separation, as shown in Table 17. It was found that by adding 0.1 per cent of gelatin, the amount of the serum layer was increased slightly, although accurate readings were difficult as this amount of the colloid gave a layer of considerably less distinctness than those in the check samples containing no gelatin. The addition of 0.2 per cent gelatin, however, caused a reduction of approximately 40 per cent in the volume of the serum layer as compared to that in the check sample, but the layer was much more distinct. Except in one trial in which a trace of a serum layer remained, it was found that adding 0.3 per cent of gelatin practically eliminated and adding 0.4 per cent of gelatin always entirely eliminated the layer.

142836—32——4

TABLE 17.—*Effect of the addition of gelatin to raw milk prior to separation*

[Average of six trials]

Gelatin added to milk	Fat in cream	Serum layer	Distinctness of serum layer	Gelatin added to milk	Fat in cream	Serum layer	Distinctness of serum layer
Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*	
None.	20.5	2.37	C+	0.3	22.5	None.	G
0.1	21.4	2.66	D−	.4	23.3	None.	G
.2	21.9	1.42	A				

EVAPORTAED MILK AND SKIM-MILK POWDER

Since it was understood that concentrated milk solids are frequently added to cream in some commercial plants in an attempt to control the serum layer in bottled cream, trials were made to determine the effect of adding evaporated milk and skim-milk powder. These were added at the rate of 0.5, 1.0, 2.0, and 5.0 per cent. The results are shown in Table 18. One-half of 1 per cent of evaporated milk had no marked influence either on the volume or on the distinctness of the serum layer. When larger amounts of evaporated milk were added, the serum layer decreased markedly both in volume and in distinctness. The addition of 5 per cent of evaporated milk eliminated the layer entirely.

The results fail to show that skim-milk powder in any of the percentages added had any influence on the size of the layer, although accurate observations were difficult when the larger amounts were added. No separation was evident in the cylinders when 5 per cent of the powder was added, but a layer was discernible in the bottled cream. It was apparent from the results that, although from 1 to 5 per cent of skim-milk powder was an aid in lessening the distinctness of the serum layer, the addition of skim-milk powder did not eliminate the serum layer entirely, as did evaporated milk when added at the rate of 5 per cent.

TABLE 18.—*Effect of the addition of evaporated whole milk, skim-milk powder, and casein to pasteurized cream containing 20.5 per cent butterfat*

Substance added	Trials	Serum layer when indicated percentage additions were made					Distinctness of serum layer when indicated percentage additions were made				
		0	0.5	1.0	2.0	5.0	0	0.5	1.0	2.0	5.0
	Number										
Evaporated whole milk	2	2.0	2.0	1.5	1.5	None.	C−	C−	D	E	G
Skim-milk powder	2	2.0	2.0	2.0	2.0	Trace.	C−	C	C−	D	E
Casein	4	1.75	------	------	------	3.88	C−	------	------	------	C+

CASEIN

The results of four trials in adding 0.5 per cent of casein indicated that casein added in this amount increased the volume of the serum layer. The check samples had on an average a 1.75 per cent layer, whereas in the samples to which casein had been added the layer averaged 3.88 per cent. There also seemed to be a tendency for the layer to be more distinct, although this effect was not pronounced.

SUMMARY AND CONCLUSIONS

From the data secured it appears that the separation of serum from bottled cream is a defect common to cream processed in the plant by the methods tested other than homogenizing or adding various substances. Separating the milk at 120° F. seemed to prevent, in part at least, the formation of a distinct serum layer, although the volume of serum layer remained practically the same irrespective of the temperature of separation. On the other hand, pasteurizing milk at 160° for 15 minutes prior to separation decreased the volume of the serum layer but had little effect upon the distinctness. Storing milk prior to separation caused no appreciable difference either in the volume or in the distinctness of the serum layer.

It appears that pasteurization alone even at as high a temperature as 160° for 30 minutes fails to prevent the formation of a distinct serum layer. However, pasteurizing raw cream at 145°, 150°, 155°, or 160° F. for 30 minutes decreased the volume and increased the distinctness of the serum layer, there being no marked difference from the results obtained at any exposure. Agitating stored pasteurized cream had no significant influence upon the volume of the serum layer, but caused it to be more distinct. The speed of cooling pasteurized cream apparently had little effect upon the volume or upon the distinctness of the serum layer.

The opinion has been current that this serum layer in bottled cream results from separating a high-fat-percentage cream and then standardizing it back to the desired richness. It seems from the data obtained that neither the method nor the heat treatment of the medium used in standardization materially reduces the volume of serum layer or lessens the intensity of its distinctness. Standardizing the cream following pasteurization appears to be desirable in eliminating, in part, the formation of a layer of serum but has little effect on its distinctness.

The length of time cream is held in storage subsequent to bottling is an important factor in the formation of the serum layer. Cream held in storage longer than 48 hours after being bottled almost invariably shows an undesirable serum layer. The volume of the serum layer increased gradually at a decreasing rate as the cream was held. The distinctness seemed to increase for some 48 to 72 hours and then gradually decreased. An increase in the temperature of creaming above 32° F. increased the volume and decreased the distinctness of the serum layer.

Viscolization at 160° F. and a pressure of 1,500 pounds per square inch appears to be equally as effective in preventing the formation of a layer of serum as 2,500 pounds pressure at 145° F. Mixing 10 per cent of cream viscolized at 145° F. with unviscolized cream reduced the layer about 44 per cent. The distinctness was not influenced to any marked extent.

The salts added gave various results. Dicalcium phosphate apparently had no influence either upon the volume or upon the distinctness of the layer. Calcium chloride, while having no apparent effect upon the volume, decreased the distinctness to some extent. The other salts added, sodium citrate, sodium carbonate, and monocalcium phosphate, apparently did not influence "creaming off" until amounts were added that partly or totally precipitated the casein.

The addition of 0.3 per cent gelatin to the milk prior to separation appears to prevent entirely the formation of a layer of serum in the cream secured. Likewise, the addition of 5 per cent evaporated whole milk to the cream entirely eliminated the serum layer. No separation was evident in the cylinders when 5 per cent skim-milk powder was added, but a layer was discernible in the bottled cream. It was apparent that although 1 to 5 per cent skim-milk powder was an aid in lessening the distinctness of the serum layer, it did not eliminate the layer entirely as when 5 per cent evaporated whole milk was added. These additions to milk and cream, although in some cases attaining the desired purpose, should be considered as adulterations, and as such are not to be recommended.

The appearance of a serum layer seems to be a defect common to most cream processed without homogenization or additions. By bottling the cream not longer than 36 to 48 hours before final distribution the formation of the serum layer is usually kept at a minimum. Pasteurizing the milk intended for skimming at 160° F. for 15 minutes, separating at 120° F., and standardizing to the desired percentage with pasteurized whole milk seems to yield a cream in which the smallest serum layer of least distinctness forms.

LITERATURE CITED

(1) Dahlberg, A. C., and Marquardt, J. C.
1929. The creaming of raw and pasteurized milk. N. Y. State Agr. Expt. Sta. Tech. Bul. 157, 80 p., illus.

(2) Farrington, E. H., and Russell, H. L.
1899. Pasteurization of milk and cream at 140° F. Wis. Agr. Expt. Sta. Ann. Rpt. 16: 129–139, illus.

(3) Hammer, B. W.
1916. Studies on the creaming ability of milk. Iowa Agr. Expt. Sta. Research Bul. 31, 91 p., illus.

(4) Judkins, H. F., and Downs, P. A.
1918. Studies in processing milk. Conn. Storrs Agr. Expt. Sta. Bul. 99, p. [449]—470.

(5) Martin, W. H., and Combs, W. B.
1924. The effect of milk plant operations on the amount of cream rising on milk. Jour. Dairy Sci. 7: 197–204, illus.

(6) Trout, G. M.
1930. Cream line studies in market milk. W. Va. Agr. Expt. Sta. Bul. 229, 20 p., illus.

(7) Webb, B. H., and Holm, G. E.
1928. The heat stability and feathering of sweet cream, as affected by different homogenization pressures and different temperatures of forewarning. Jour. Dairy Sci. 11: 243–257, illus.

(8) Whittaker, H. A., Archibald, R. W., Sheve, L., and Clement, C. E.
1925. Effect of various factors on the creaming ability of market milk. U. S. Dept. Agr. Bul. 1344, 24 p., illus.

INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN HYBRIDS OF WHITE FEDERATION AND ODESSA WHEAT [1]

By FRED N. BRIGGS
Assistant Agronomist, California Agricultural Experiment Station

INTRODUCTION

The inheritance of resistance to bunt, *Tilletia tritici* (Bjerk.) Wint., has been reported for hybrids between susceptible varieties of wheat and the resistant varieties Martin (*1*),[2] Hussar (*1*, *2*), White Odessa (*3*), and Banner Berkeley (*4*). The present paper deals with the inheritance of resistance to bunt in hybrids between susceptible White Federation and resistant Odessa wheats. Data are also included from Martin × White Odessa which were not available for the earlier publication on White Odessa (*3*).

METHODS AND MATERIALS

The parental material and hybrid populations were grown in the field at University Farm, Davis, Calif. The methods of handling and the inoculum used have been described in previous publications (*1*, *2*, *3*, *4*).[3] This bunt has been designated as physiologic race III of *Tilletia tritici*, by Reed (*7*).

The resistant parent was obtained in 1919 under the name of Goldcoin but was later identified as Odessa, by J. A. Clark, Division of Cereal Crops and Diseases, United States Department of Agriculture.

That Odessa is very resistant as compared with White Federation may be seen in Table 1.

TABLE 1.—*Annual percentage of bunt infection in the parent wheat varieties*

Variety	Percentage of bunted plants in—							
	1920	1921	1922	1927	1928	1929	1930	1931
Odessa	0.6	0	0.7	0		0	0	0
Martin	0	0	0	0	0	0	0	0
White Federation	88.7	51.6	58.3	66.6	68.9	78.6	62.2	44.9

It is not known just why Odessa produced a little bunt during 1920 and 1922 but has been bunt free in other years when grown under similar conditions and inoculated with bunt from the same original source. Similar results were obtained with White Odessa (*3*) and Banner Berkeley wheats (*4*). Such small amounts of bunt are of little importance. Martin has been completely resistant during the entire period.

[1] Received for publication Dec 21, 1931; issued October, 1932.
[2] Reference is made by number (italic) to Literature Cited, p. 505.
[3] BRIGGS, F. N. INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN CROSSES OF WHITE FEDERATION WITH TURKEY 1558 AND TURKEY 3055. Unpublished manuscript.

EXPERIMENTAL RESULTS

Some of the F_2 seeds of all crosses were treated with copper carbonate to protect them from bunt infection in order to grow seed for the F_3 generation. The rest of the F_2 seeds were inoculated with bunt and grown the following year along with the F_3 rows.

F_2 data do not permit a satisfactory Mendelian analysis because some susceptible plants usually escape infection. Also heterozygous resistant and homozygous resistant plants occasionally may become infected. F_2 data do indicate, however, the percentage of bunt to expect in F_3 rows of the same genotype. Accordingly, the results are given in Table 2.

TABLE 2.—*Percentage of bunted plants in the parents and F_2 of the crosses named when grown in the field at Davis, Calif.*

Parent or cross	Year grown	Plants grown	Plants bunted	
		Number	*Number*	*Per cent*
Odessa	1930	412	0	0
Martin	1930	141	0	0
White Federation	1930	546	334	61.2
F_2 White Federation × Odessa	1930	1,161	175	15.1
F_2 Martin × Odessa	1930	658	0	0
White Odessa	1931	143	0	0
Martin	1931	159	0	0
White Federation	1931	648	291	44.9
F_2 Martin × White Odessa	1931	359	0	0

There was an average of 15.1 per cent of bunted plants in the F_2 of White Federation × Odessa, which is considerably less than the 25 per cent expected on the basis of a single dominant factor for resistance. Martin × White Federation produced 17.2 per cent of bunted plants in F_2 (*1*). At that time it was shown that enough susceptible plants had escaped infection to bring this figure into satisfactory agreement with the 25 per cent expected. The F_2 data, then, indicate that Odessa differs from White Federation in a single dominant factor for resistance to bunt, and that heterozygous F_3 rows should contain an average of about 15 per cent of bunt.

TABLE 3.—*Distribution of parent and F_3 rows of the crosses named into 5 per cent classes for bunt infection, when grown at Davis, Calif.*

Parent or cross	Year grown	Distribution of rows of wheat plants having a bunt infection (per cent) of—																			Total number of rows
		0	1 to 5	5 to 10	10 to 15	15 to 20	20 to 25	25 to 30	30 to 35	35 to 40	40 to 45	45 to 50	50 to 55	55 to 60	60 to 65	65 to 70	70 to 75	75 to 80	80 to 85	85 to 90	
White Federation	1930												3	3	2	2	2				12
Odessa	1930	10																			10
Martin	1930	4																			4
White Federation × Odessa	1930	51	18	14	42	50	29	7	4	8	2	13	17	10	9	14	6	6	1	1	302
Martin × Odessa	1930	149																			149
Martin	1931	4																			4
White Odessa	1931	4																			4
White Federation	1931								3	1	4	5	3	2	1	1					20
Martin × White Odessa	1931	98																			98

The F_2 of Martin × Odessa was free from bunt, indicating that Odessa has the same factor for resistance to bunt as Martin F_2 data of Martin × White Odessa indicate that White Odessa also has the Martin factor.

Obviously, the classification of F_2 plants on the basis of the behavior of their progeny in F_3 rows is more reliable than classification in F_2. F_3 rows contained from 30 to 60 plants. These data are recorded in Table 3.

The rows having from 0 to just less than 5 per cent of bunted plants were separated into those with no bunted plants and those with some but less than 5 per cent of bunted plants, because of the special interest in the former. The nature of the distribution may be seen more readily in Figure 1. The numbers of rows under the three modes are in close agreement with the 1:2:1 ratio. As in the crosses with Martin, White Odessa, and Banner Berkeley the first minimum fell at

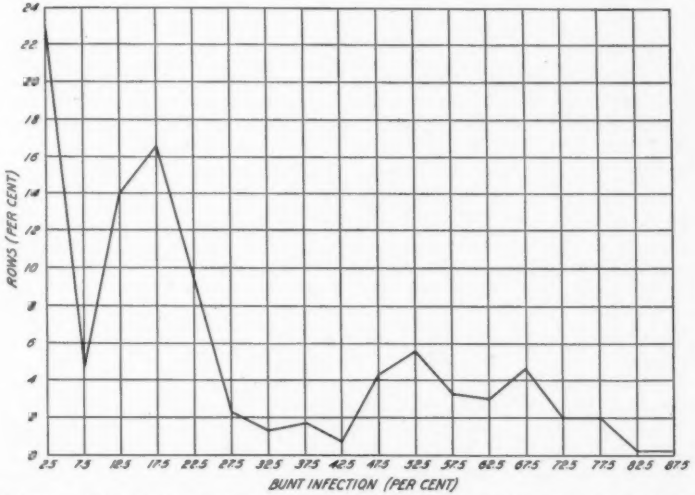

FIGURE 1.—Distribution of F_3 rows of the cross White Federation × Odessa into 5 per cent classes of bunt infection

7.5 per cent of bunted plants. The division between the heterozygous and the susceptible rows is not so sharp but has been somewhere near 50 per cent of bunt infection, falling at 42.5 in this case. As divided by these minima there were 76 resistant, 148 heterozygous, and 78 susceptible rows where 75.5, 151.0, and 75.5, respectively, were expected. The susceptible rows had an average of 60.1 per cent of bunt while 12 rows of White Federation averaged 62.2 per cent. The heterozygous rows averaged 18.5 per cent under the same conditions under which 30 F_2 rows produced 15.1 per cent of bunted plants.

No doubt some rows are classified incorrectly by accepting these minima as the points of division between phenotypes. Duplicate plantings were made of 53 F_3 rows and their classification was compared, using the minima established by the entire F_3 as the points of division between phenotypes. The second planting was made in the same nursery but at some distance from the first. The results are shown in Table 4.

TABLE 4.—*Percentage of bunted plants in duplicate plantings of F_3 row of White Federation × Odessa*

Row No.	Percentage of bunt		Row No.	Percentage of bunt		Row No.	Percentage of bunt		Row No.	Percentage of bunt	
	Series 1	Series 2		Series 1	Series 2		Series 1	Series 2		Series 1	Series 2
1	48.3	67.5	15	1.6	0	28	54.8	60.3	41	20.3	17.5
2	0	2.3	16	17.2	11.3	29	16.7	19.6	42	51.2	43.6
3	0	0	17	14.3	14.8	30	0	2.0	43	21.0	21.3
4	23.1	11.8	18	3.9	1.7	31	20.0	14.0	44	0	0
5	66.7	53.2	19	47.5	52.5	32	19.6	22.8	45	58.5	57.4
6	33.3	45.3	20	54.3	61.8	33	17.1	29.6	46	0	0
7	7.8	20.0	21	66.7	53.8	34	60.0	54.7	47	20.4	9.3
8	16.4	13.5	22	23.5	14.8	35	25.5	23.6	48	13.0	29.3
9	0	0	23	12.7	27.5	36	0.0	1.7	49	12.8	21.3
10	12.3	18.6	24	1.7	3.5	37	15.6	7.3	50	25.0	18.5
11	13.7	22.4	25	68.4	67.2	38	18.2	19.1	51	35.5	10.9
12	16.7	9.8	26	0	0	39	0	0	52	0	0
13	14.1	15.5	27	27.5	8.8	40	2.4	2.7	53	14.3	20.0
14	49.1	44.8									

In a number of cases the actual percentages of bunt vary considerably between series 1 and series 2, but in many cases they are in close agreement. The average percentage of bunt in the first series is 21.9 and in the second 21.7. The classification in the second series differs from the first in only two cases. No. 6 changed from heterozygous to susceptible and No. 37 from heterozygous to resistant. A similar experiment was made with 36 F_3 rows of White Federation × Banner Berkeley. The data are very similar to those in Table 4, even though the first planting was made in 1929 and the duplicate planting in 1930. The classification differed in only one case. No. 28 was classified as heterozygous in 1929, but as resistant with 7.3 per cent of bunt in 1930. These duplicate plantings indicate that the minima indicate the division between phenotypes quite accurately.

The classification of F_2 plants, determined on the basis of the percentage of bunted plants in F_3 rows, shows that Odessa differs from White Federation in one main dominant factor for resistance to bunt. That this factor is identical with the factor in Martin wheat is evidenced by the fact that there was no bunt in the F_2 or 149 F_3 rows of the Martin × Odessa cross.

In an earlier publication (*3*) it was shown that White Odessa differed from White Federation in one main dominant factor for resistance to bunt, which was similar in its effect to the Martin factor. The factor for resistance to bunt in White Odessa is identical with the factor in Martin.

DISCUSSION AND SUMMARY

The literature discussed in earlier publications will not be repeated here. Recently, Churchward (*5*) has reported that Florence differs from Hard Federation in one main factor for resistance to bunt. This is of special interest because Florence is a parent of Ridit, which is very resistant to bunt, and was selected from a cross of Turkey × Florence, by Gaines (*6*). The writer [4] has found that Turkey differs from White Federation in one main factor for resistance. Unless there are some factors in Turkey or Florence which are not apparent

[4] BRIGGS, F. N. Op. cit. (See footnote 3.)

in the presence of the physiologic forms of bunt used in these experiments, Ridit should not contain more than these two factors for resistance to bunt. However, unpublished data from crosses of Florence × White Federation and Florence × Big Club, grown at this station, have yielded data which the writer has not been able to analyze satisfactorily for Mendelian ratios. This may indicate the presence of a large number of modifying factors or possibly multiple factors. These points are being investigated. A difference in bunt might give different results. The bunt used at Davis is the one designated as physiologic race III of *Tilletia tritici*, by Reed (7).

Odessa and White Odessa, like Martin and Banner Berkeley, may be designated as *M M h h*. Hussar is of the *M M H H* constitution. The proper designation for Turkey 1558 and Turkey 3055 is not known at present. They each contain a single factor for resistance which is similar in effect to the second Hussar factor *H H*.

LITERATURE CITED

(1) Briggs, F. N.
1926. INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI (BJERK.) WINTER, IN WHEAT. Jour. Agr. Research 32:973–900, illus.
(2) ———
1930. INHERITANCE OF THE SECOND FACTOR FOR RESISTANCE TO BUNT, TILLETIA TRITICI, IN HUSSAR WHEAT. Jour. Agr. Research 40:225–232, illus.
(3) ———
1930. INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN WHITE ODESSA WHEAT. Jour. Agr. Research 40:353–359, illus.
(4) ———
1931. INHERITANCE OF RESISTANCE TO BUNT, TILLETIA TRITICI, IN HYBRIDS OF WHITE FEDERATION AND BANNER BERKELEY WHEATS. Jour. Agr. research 42:307–313, illus.
(5) Churchward, J. G.
1931. STUDIES IN THE INHERITANCE OF RESISTANCE TO BUNT IN A CROSS BETWEEN FLORENCE × HARD FEDERATION WHEATS. Roy. Soc. N. S. Wales, Jour. and Proc. 64:298–319, illus.
(7) Gaines, E. F.
1923. GENETICS OF BUNT RESISTANCE IN WHEAT. Jour. Agr. Research 23:445–480, illus.
(7) Reed, G. M.
1928. PHYSIOLOGIC FACES OF BUNT OF WHEAT. Amer. Jour. Bot. 15:157–170.

THE EFFECT OF ARTIFICIAL DRYING ON THE AVAILABILITY OF THE NUTRIENTS OF ALFALFA HAY[1]

By E. B. HART, *Professor of Agricultural Chemistry*, O. L. KLINE, *Assistant in Agricultural Chemistry*, and G. C. HUMPHREY, *Professor of Animal Husbandry*, *Wisconsin Agricultural Experiment Station*[2]

INTRODUCTION

Recently there have come into practical use a number of driers designed for the rapid desiccation of large quantities of green plant tissue. Naturally the question of the availability of the nutrients of such dried hays as compared with those of hays cured in the ordinary way becomes important.

One of the first driers used in this country for the desiccation of green plants was the Mason drier. In this machine the period of drying is from 30 to 35 minutes, and the temperature of the heated air as it passes from the furnace to the drying tunnel is 127° to 129° C. Later the Koon drier came into use. A Koon drier, established on the farm of Howard T. Green at Genesee Depot, Wis., was used there experimentally for a number of years. The maximum temperature to which plants were exposed in the process of drying and the time of exposure were definitely measured. The time consumed in the drying process was not more than one minute. The highest temperature recorded for fresh green material was 480° to 535° C. with an exposure of 40 seconds. Where the material had already been partly dried lower temperatures were used at the inlet. Such high temperatures of the drying gases must be used in the evaporation of the water without subjecting the dry matter itself to them. In spite of the high temperatures the hay comes out of the drier retaining a pea-green color. It was not known, however, whether these temperatures had rendered unavailable certain valuable constituents of the plants, and it was to determine the effects of this method of drying on the availability of important nutrients that the experiment herein reported was undertaken.

Studies have been carried out on the effect of the Mason drying process on the vitamin A and D content of alfalfa.[3] Russell found that alfalfa cured by the Mason process was seven times as potent in its vitamin A content as field-cured hay, but that it was very low in vitamin D—as would be expected when compared with alfalfa cured in the sun. Hauge and Aitkenhead[4] also found that alfalfa dried in artificial driers, at a much higher temperature than that used in the Mason drier, was still rich in vitamin A. They say: "Even a temperature as high as the hot flue gas used in one of the machines, or the sterilization temperature of the autoclave, was not destructive." No mention is made of the type of drier used.

---

[1] Received for publication Mar. 4, 1932; issued October, 1932.
[2] The writers' thanks are due Howard T. Green, of Brook Hill Farms, Genesee Depot, Wis., for making this investigation possible.
[3] RUSSELL, W. C. THE EFFECT OF THE CURING PROCESS UPON THE VITAMIN A AND D CONTENT OF ALFALFA. Jour. Biol. Chem. 85: 289–297. 1929.
[4] HAUGE, S. M., and AITKENHEAD, W. THE EFFECT OF ARTIFICIAL DRYING UPON THE VITAMIN A CONTENT OF ALFALFA. Jour. Biol. Chem. 93: 663. 1931.

EXPERIMENTAL PROCEDURE

Three lots of second-growth alfalfa hay, cut from the same field were prepared at the Brook Hill farm. The hays were cured in three different ways: Hay No. 1 was cut on August 1, 1930, and dried immediately in the Koon drier. Hay No. 2 was cut at 2.30 p. m., July 31, raked into a windrow late in the afternoon, hauled just before noon on August 1, and dried in the artificial drier. This hay had lost about 40 per cent of its moisture before it was actually dried in the drier. Hay No. 3 was field cured, cut on August 13, at 3 p. m., raked into windrows on August 14 about 1 p. m., and cocked about 4.30, August 14. This hay was hauled August 20, cut with a silage cutter, and blown into bags.

The hays, cut and bagged, were delivered to the experiment station. All were a rich pea-green color. Studies on the digestible dry matter, digestible protein, and available calcium were made with three high-producing Holstein cows that were in the early part of their third lactation period. The metabolism experiment was begun February 5, 1931. Cow No. 1 freshened January 15, 1931; cow No. 2 freshened December 22, 1930; and cow No. 3 freshened December 20, 1930. All developed into heavy milkers—that is, they produced 50 to 60 pounds of milk daily.

The ration used consisted of 10 pounds of the alfalfa hay, 25 pounds of corn silage, and for every 3 to 3½ pounds of milk 1 pound of a grain mixture made of 59 parts of yellow corn, 25 parts of wheat bran, 15 parts of oil meal, and 1 part of salt.

In the first period of three weeks the animals were fed hay 1 (dried immediately); in the second period of three weeks, hay 2 (partly field cured and then dried in the drier); and in the third period of three weeks, hay 3 (sun cured in the field).

Quantitative collection of the excreta and quantitative collection of the milk, with a complete analysis of income and outgo, particularly in reference to calcium, digestible dry matter, and digestible nitrogen, were made. During the course of the experiment the feed consumption by the animals was complete, except in the ninth week, when cow 2 left some residue.

EXPERIMENTAL DATA

Table 1 gives the record of digestibility of the dry matter. A study of this table indicates that the coefficients of digestibility of the dry matter of the three hays were very much alike. Apparently drying at a high temperature for 40 seconds had in no way impaired the availability of the dry matter of alfalfa for the cow.

TABLE 1.—*Data on digestibility of dry matter in alfalfa dried and cured in different ways*

PERIOD 1—HAY 1 (ALFALFA DRIED IMMEDIATELY)

Week No.	Cow 1				Cow 2				Cow 3			
	Dry matter intake	Dry matter in feces	Difference	Coefficient of digestibility	Dry matter intake	Dry matter in feces	Difference	Coefficient of digestibility	Dry matter intake	Dry matter in feces	Difference	Coefficient of digestibility
	Grams	*Grams*	*Grams*	*Per ct.*	*Grams*	*Grams*	*Grams*	*Per ct.*	*Grams*	*Grams*	*Grams*	*Per ct.*
1	91,286	26,461	64,825	71.0	88,516	25,558	62,958	71.1	91,286	27,187	64,099	70.2
2	91,286	25,530	65,756	72.0	88,516	24,948	63,568	71.8	91,286	28,093	63,193	69.2
3	91,286	27,179	64,107	70.2	88,516	24,617	63,899	72.1	91,286	28,745	62,541	68.5
Average				71.0				71.7				69.3
PERIOD 2—HAY 2 (ALFALFA PARTLY CURED)												
4	90,427	26,713	63,714	70.4	87,662	26,617	61,045	69.6	90,427	29,731	60,696	67.1
5	90,427	25,764	64,664	71.5	87,662	23,976	63,686	72.6	90,427	28,080	62,347	68.9
6	90,427	26,235	64,192	71.0	87,662	26,114	61,548	70.2	90,427	27,044	63,383	70.0
Average				71.0				70.8				68.7
PERIOD 3—HAY 3—(ALFALFA SUN CURED)												
7	90,538	24,646	65,892	72.7	87,784	25,017	62,767	71.5	90,538	27,294	63,244	69.8
8	90,538	27,113	63,425	70.0	87,784	26,840	60,944	69.4	90,538	28,442	62,056	68.5
9	90,538	26,309	64,229	70.9	87,784	18,688	69,096	(a)	90,538	28,189	62,349	68.9
Average				71.2				70.4				69.1

[a] Cow off feed.

Table 2 gives a record of the digestible nitrogen. A careful study of this table shows that the protein and other nitrogenous complexes of the hay were not rendered unavailable through the heat treatment of this particular drier. One might interpret the records of cows 1 and 3 as indicating a slightly lower availability of the protein in the hay that was immediately dried as compared with that in the sun-cured hay, but the differences are too slight to be of any real significance. In the case of cow 2 the availability of the nitrogen in the three periods was practically identical.

TABLE 2.—*Data on digestibility of nitrogen in alfalfa dried and cured in different ways*

PERIOD 1—HAY 1 (ALFALFA DRIED IMMEDIATELY)

Week No.	Cow 1				Cow 2				Cow 3			
	Total nitrogen intake	Nitrogen in feces	Nitrogen digested	Coefficient of digestibility	Total nitrogen intake	Nitrogen in feces	Nitrogen digested	Coefficient of digestibility	Total nitrogen intake	Nitrogen in feces	Nitrogen digested	Coefficient of digestibility
	Grams	*Grams*	*Grams*	*Per ct.*	*Grams*	*Grams*	*Grams*	*Per ct.*	*Grams*	*Grams*	*Grams*	*Per ct.*
1	2,315.95	642.60	1,673.35	72.2	2,229.85	666.60	1,563.25	70.1	2,315.95	773.14	1,542.81	66.6
2	2,315.95	612.70	1,703.25	73.1	2,229.85	631.80	1,598.05	71.6	2,315.95	719.96	1,595.99	68.9
3	2,315.95	639.00	1,676.95	72.4	2,229.85	640.80	1,589.05	71.3	2,315.95	827.68	1,488.27	64.3
Average				72.6				71.0				66.6
PERIOD 2—HAY 2 (ALFALFA PARTLY CURED)												
4	2,379.51	598.20	1,781.31	74.9	2,293.41	686.88	1,606.53	70.0	2,379.51	798.75	1,580.76	66.5
5	2,379.51	639.00	1,740.51	73.3	2,293.41	642.82	1,650.59	71.9	2,379.51	793.49	1,586.02	66.6
6	2,379.51	613.80	1,765.71	74.2	2,293.41	645.84	1,647.57	71.8	2,379.51	685.26	1,694.25	71.2
Average				74.1				71.2				68.1
PERIOD 3—HAY 3 (ALFALFA SUN CURED)												
7	2,363.62	564.60	1,799.02	76.1	2,277.52	652.15	1,625.37	71.3	2,363.62	772.74	1,590.88	67.2
8	2,363.62	591.00	1,772.62	75.0	2,277.52	637.81	1,639.71	72.0	2,363.62	773.26	1,590.36	68.9
9	2,363.62	552.60	1,811.02	76.6	2,277.52	395.14	1,882.38	(a)	2,363.62	748.50	1,615.12	68.3
Average				75.9				71.6				68.1

[a] Cow off feed.

In Table 3 are given the records of the calcium metabolism of the cows during the three periods, as well as the weekly milk production. As is usually the case with cows in the early part of lactation, especially liberal producers, these animals are in negative lime balance. The results recorded here are in harmony with previous observations in calcium metabolism work with cows. There is no indication, however, that the different treatments to which the hays had been subjected materially influenced the availability of the calcium. The calcium in the immediately dried hay appears to have been a little more readily assimilated, but the difference is very slight.

TABLE 3.—*Calcium balance and milk production of three cows when fed alfalfa hay which had been dried and cured in different ways*

COW 1—PERIOD 1—HAY 1 (DRIED IMMEDIATELY)

Week No.	CaO in feces	CaO in milk	CaO in urine	Total CaO excreted	Total CaO intake	CaO balance	Weekly milk production
	Grams	*Grams*	*Grams*	*Grams*	*Grams*		*Pounds*
1	708.29	251.57	7.59	967.45	895.28	−72.17	432.8
2	699.93	246.49	15.61	962.03	895.28	−66.75	448.7
3	733.28	233.96	10.49	977.73	895.28	−82.45	422.4
COW 1—PERIOD 2—HAY 2 (PARTLY SUN CURED)							
4	677.06	251.39	9.96	938.41	803.71	−134.70	436.0
5	672.95	254.82	9.95	937.72	803.71	−134.01	412.7
6	669.17	250.43	6.50	926.10	803.71	−122.39	427.6

TABLE 3.—*Calcium balance and milk production of three cows when fed alfalfa hay which had been dried and cured in different ways*—Continued

Week No.	CaO in feces	CaO in milk	CaO in urine	Total CaO excreted	Total CaO intake	CaO balance	Weekly milk production
	Grams	*Grams*	*Grams*	*Grams*	*Grams*		*Pounds*
COW 1—PERIOD 3—HAY 3 (SUN CURED)							
7	623.51	233.57	16.04	873.12	753.50	−119.62	414.9
8	669.93	221.03	11.66	902.62	753.50	−149.13	405.7
9	636.63	193.41	15.44	845.48	753.50	−91.98	377.0
COW 2—PERIOD 1—HAY 1 (DRIED IMMEDIATELY)							
1	689.56	248.96	9.83	948.35	890.59	−57.76	340.6
2	661.23	248.01	10.70	919.94	890.59	−29.35	333.1
3	614.63	246.74	7.82	869.19	890.59	+21.40	323.5
COW 2—PERIOD 2—HAY 2 (PARTLY SUN CURED)							
4	628.21	243.83	8.49	880.53	798.43	−82.10	317.8
5	593.57	247.49	7.63	848.69	798.43	−50.26	313.3
6	611.58	231.43	9.82	852.83	798.43	−54.40	318.6
COW 2—PERIOD 3—HAY 3 (SUN CURED)							
7	637.53	231.40	10.71	879.64	748.22	−131.42	301.6
8	583.55	222.90	18.07	824.52	748.22	−76.30	294.0
9	400.78	149.28	6.65	556.71	748.22	[a]+191.51	196.9
COW 3—PERIOD 1—HAY 1 (DRIED IMMEDIATELY)							
1	635.22	271.46	10.98	917.66	895.28	−22.38	427.1
2	634.68	249.07	8.55	892.30	895.28	+2.98	428.6
3	660.05	266.67	11.79	938.51	895.28	−43.23	435.1
COW 3—PERIOD 2—HAY 2 (PARTLY SUN CURED)							
4	662.44	239.28	5.47	907.19	803.71	−103.48	428.5
5	623.66	294.92	7.59	926.17	803.71	−122.46	430.2
6	553.28	271.31	7.75	832.34	803.71	−28.63	436.2
COW 3—PERIOD 3—HAY 3 (SUN CURED)							
7	601.28	263.70	5.41	870.39	753.50	−116.89	420.9
8	612.06	265.59	5.89	883.54	753.50	−130.04	414.9
9	582.63	229.05	7.35	819.03	753.50	−65.53	388.1

[a] Cow off feed.

SUMMARY

Second-cutting alfalfa hays, (1) dried immediately in an artificial drier (Koon); (2) partly dried in the field and then dried in the artificial drier, and (3) completely dried in the field, were studied for the availability of their dry matter, protein, and calcium.

The availability of the dry matter, protein, and calcium was much alike in all three hays, as measured by metabolism experiments with heavy-milking cows.

Apparently the high temperature of 480° to 535° C. for 40 seconds, to which the green legume plants were exposed in the immediate-drying process, did not reduce the availability of the nutrients studied.

U. S. GOVERNMENT PRINTING OFFICE: 1932